Optim® 1000

A unique high throughput, micro-volume protein analysis and characterisation system.

User manual

v1.5.4

Avacta™ ANALYTICAL

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Avacta Analytical Optim 1000 v1.5

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HOW TO USE THIS MANUAL

Welcome to the Optim 1000 Installation and Operation Manual. This section, believe it or not, is an instruction manual for your instruction manual. There are some useful pointers here so don't despair! Take a minute to read this and the rest of the document will be easier to interpret.

Software Instructions

Avacta's software provides the human-machine interface. You will soon learn to navigate it by instinct; but until you find your feet, some things to look out for in the software have been highlighted for you using the following code:

Bold words indicate a **tab** or **menu.**

Italics indicate a *Menu Option*.

CAPITALS indicate a BUTTON.

- Direct instructions to the user are bullet-pointed for clarity, so you can scan the text and skip over the explanations if you wish.

TIP – This symbol is used to inform the user of a shortcut or toolbar button

Glossary

At the end of the guide there is a **Glossary of Terms** which explains all of the jargon that appears in this guide. Throughout the guide text, a word that has a specific meaning for the Optim 1000 software for example a tab heading, is indicated by a capital letter at the start (for example, Project, Experiment, Run). It is advisable to look through the Glossary before beginning to use the instrument.

Index

To find information relating to a particular tab or topic, refer to the Index at the end of this guide.

Instrument handling

Watch out for the following alert words in the text, they will advise you of a physical hazard or an important step for you to carry out. The alert words indicate the particular level of observation or action, as defined below:

IMPORTANT – Indicates information that is necessary for proper instrument operation or safe usage.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also alert against unsafe practices.
WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This is limited to only the most extreme situations.

PREPARATION

I. SITE PREPARATION

This section of the manual describes how to prepare your site for installation of the Optim 1000 and how to get the instrument up and running. There are important safety warnings included here which must be observed by all personnel that have access to the instrument. Hardware installation should be completed before beginning the software.

You should not unpack the instrument until the installation engineer is present. An Avacta Analytical qualified engineer should guide you through the site preparation and installation of the Optim 1000, so the following section is for information only.

Before installing the Optim 1000 in the workplace, you need to prepare the site for installation according to the guidelines in this section. At the end of the section there are a set of checklists to help ensure that all the preparation tasks have been addressed.

IMPORTANT - If the Optim Instrument is moved after it has been installed, it must be re-qualified by an Avacta service engineer before use.

System Components

The Optim 1000 consists of the following items:

- Main Optim 1000 unit
- External PC with keyboard and mouse
- Monitor
- Connector cables

Dimensions and weights

The dimensions and weights of a typical Optim 1000 system are given below. Ensure that the installation site can accommodate the dimensions and weights.

Component	Width	Depth	Height	Weight
Optim unit	85 cm (34")	69 cm (28")	76 cm (30")	75 kg (165 lb)
Desktop PC	17 cm* (7")	43 cm* (17")	37 cm* (15")	10 kg* (22 lb)
Monitor & Keyboard	Standard size			

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Clearances

Once in operation, the main Optim 1000 unit needs at least 15 cm (6 inches) clearance on all sides for ventilation.

For installation and on-site repair and maintenance, the Engineer will require at least 60 cm (24 inches) clearance either side of the instrument to gain access to the side panels.

Cleaning your Optim

Should you wish to clean your instrument after installation, you must first follow the shutdown procedure and switch off the unit at the mains. Wipe the exterior with a soft dry cloth or if necessary, a damp cloth with mild detergent.

Environmental Requirements

The site should be maintained under the following conditions.

The user should be aware that fluctuations in environmental conditions can cause unexpected changes in the instrument's performance. For best results, ensure that the temperature and humidity are maintained at a constant level.

Condition	Acceptable Range
Temperature	15 to 30 °C (50 to 95 °F)
Humidity	20 to 80 % relative humidity, non condensing

This apparatus is for indoor use only. Under normal operation it has ingress protection rating IP20. The instrument should not be placed in direct sunlight.

Electrical Requirements

The main Optim 1000 unit requires connection to one standard mains socket.

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The Desktop PC requires connection to one mains socket. The monitor may require connection to one mains socket (or it may plug into the desktop PC). The Desktop PC will have its own standard power requirements.

> ⚠ **IMPORTANT -** In case of emergency, you must be able to immediately disconnect the main power supply to the instrument.

The main Optim 1000 unit is factory configured to operate interchangeably at ~120 VAC or ~240 VAC.

Voltage	Frequency	Current drawn for Power consumed	Fuse Rating	Max. Power
220-240 V	50-60 Hz	0.5 – 1 A	2 A	0.15 kW (VA)
110-125 V	50-60 Hz	1.25 – 2.5 A	4 A	0.15 kW (VA)

Over current protection >110%

Checklists

Check that the location fulfils the following criteria:

- ☑ The bench or table area is sufficiently large and can withstand the weights of the instrument.
- ☑ There are sufficient mains power sockets within range.
- ☑ The location of the instrument and computer allows for appropriate ergonomic usage.

Check that all the following components are present:

- ☑ Optim 1000 analytical instrument
- ☑ Mains power lead
- ☑ USB lead
- ☑ Ethernet lead
- ☑ Desktop PC
- ☑ Monitor
- ☑ Mouse

If your installation site is suitable and all of the Optim 1000 components are present, you are almost ready to begin! Your installation Engineer will set up the instrument for you so in the meantime, take the time to read the important safety information in

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the following section so that you can look out for those potential hazards when you begin to operate the instrument.

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II. SAFETY ADVICE

For your protection and to avoid malfunction of your Optim 1000, observe the following safety precautions. Read and understand all the information in the instructions for use and observe all warning and caution statements. Failure to do so may result in damage to the product, injury to personnel or poor instrument performance.

The following table describes the symbols that may appear on Optim instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard.

Symbol	Description
	Indicates that you should consult the manual for further information and proceed with caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser hazard and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).

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General Instrument Safety

The Optim 1000 was designed with due consideration of the Low Voltage, Electromagnetic Compatibility and Machinery Directives (2006/95/EC, 2004/108/EC and 98/37/EC respectively). It has been independently tested to EN61010-1:2001, EN61010-2-81:2002, EN 61326-1:2006, EN55022:2006 + A1:2007, EN5502:1998+A1:2001 +A2 2003, EN61000-3-2:2006, EN61000-3-2:2006, EMC-FCC: Title 47 of the CFR:2008 Part 15(b) (EMC requirements).

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful Interference in which case the user will be required to correct the interference at his own expense.

Please be aware that any changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment. (FCC CFR 47 section 15.21).

Moving and lifting the Instrument

CAUTION - Physical Injury Hazard. Do not attempt to move or lift the instrument without the assistance of others, the use of appropriate moving equipment and proper lifting techniques.

Improper lifting can result in painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

IMPORTANT If the Optim Instrument is moved after it has been installed, it must be re-validated by an Avacta qualified service engineer before use.

Moving and Lifting Stand-Alone computers and Monitors

CAUTION - Physical Injury Hazard. Do not attempt to move or lift the computer or monitor without the assistance of others. Depending upon the weight of the computer and/or the monitor, moving them may require two or more people.

- Make sure you have a secure, comfortable grip on the computer or monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.

- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate with each other before and during lifting objects.

Ensure that everyone who operates the instrument has received instructions in general safety practices for laboratories and specific practices for the instrument.

Electrical Safety

The Optim 1000 instrument is classified as portable equipment.

DANGER – Electrical Shock Hazard. Severe electric shock can result from operating the instrument without its panels in place. Do not remove the instrument panels. High voltage contacts are exposed when the instrument panels are not in place.

Power

DANGER – Electrical Hazard. Use properly configured and approved line cords for the local voltage supply.

DANGER – Electrical Hazard. Continuous grounding circuitry is vital for safe operation of equipment. Never operate the instrument with grounding conductors disconnected.

DANGER – Electrical Hazard. Plug the system into a properly grounded receptacle with adequate current capacity.

Fuses

WARNING – Fire Hazard. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the local power supply.

DANGER – Fire Hazard. Replace fuses only with fuses of the type and rating specified for the instrument.

Laser Safety

CAUTION – Laser Hazard Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Optim is a Class I laser product. The lasers within the instrument have the following specifications.

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Wavelength	Power output	Pulse width/ Repetition rate	Laser class
266 nm	< 15 mW	1 ns/ ~6-10 kHz	Class 4
473 nm	< 20 mW	CW	Class 3B

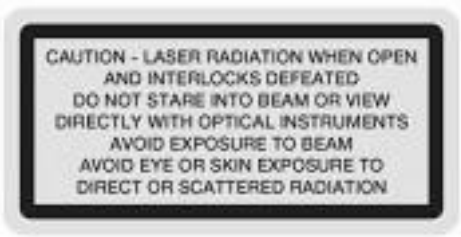
Danger to Eyes

DANGER – Laser Hazard. Direct or indirect exposure of a laser beam to the eyes may result in permanent damage to the eyes, including photokeratitis, photochemical cataracts and retinal injuries. If operating the instrument without its covers, always wear appropriate laser protective eyewear.

Hazard to Skin

WARNING – Physical Injury Hazard. Direct exposure of a laser beam to the skin may cause erythema, melanoma, accelerated skin aging, increased pigmentation and photosensitive reactions. If operating the instrument without its covers, always wear appropriate personal protective equipment (gloves).

Laser Warning Labels


CAUTION - LASER RADIATION WHEN OPEN
AND INTERLOCKS DEFEATED
DO NOT STARE INTO BEAM OR VIEW
DIRECTLY WITH OPTICAL INSTRUMENTS
AVOID EXPOSURE TO BEAM
AVOID EYE OR SKIN EXPOSURE TO
DIRECT OR SCATTERED RADIATION

This label is displayed on the top of each of the left and right side panels of the instrument.

Physical Hazards

Moving Parts

WARNING – Physical Injury Hazard. Moving parts can crush and cut. Keep hands clear of moving parts, including the motorised door, while servicing or operating the instrument.

Hot Plate

WARNING – Physical Injury Hazard. The plate where the samples are located may become hot during operation. Allow sufficient time for it to cool before moving or changing the samples.

Workstation Safety

Correct ergonomic configuration of the workstation can reduce or prevent effects such as fatigue, pain and strain. Minimise or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

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CAUTION – Musculoskeletal and Repetitive Motion Hazard. These hazards are caused by repetitive motion, awkward posture, holding static unhealthy positions, contact pressure and other workstation environmental factors.

To minimize risks, use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor and mouse.

Chemical Safety

Chemical Hazard Warning

WARNING – Chemical Hazard. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer and observe all relevant precautions.

WARNING – Chemical Hazard. Chemicals in the instrument (including liquid in the lines) are potentially hazardous.

Chemical manufacturers supply current **Material Safety Data Sheets (MSDSs)**. These contain information on storing, handling, transporting and disposing of the chemicals safely.

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle or work with any chemicals or hazardous materials.
- Minimise contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, protective clothing).
- Minimise the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures.
- Comply with all local or national laws and regulations related to chemical storage, handling and disposal.

Biological Safety

General Biohazard

WARNING – Biohazard. Your samples may contain components that have potential to transmit infectious diseases. Follow all appropriate local and national regulations. Wear appropriate protective eyewear, gloves and clothing when handling such samples.

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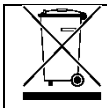
- The user must take responsibility for handling their samples in an appropriate and safe manner. The samples are contained with the MCA sample holders that are loaded into Optim. The samples should remain contained within these sample holders throughout the course of the experiments.
- Once removed from Optim, the samples and sample holders should be disposed of in an appropriate manner.

Disposal



Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).

- If the Optim unit is removed for disposal, the user should dispose of it in an appropriate manner. The Optim unit is made primarily from standard metal, plastic and electronic components. The WEEE regulations allow the onus for disposal of such equipment to reside with the user.

The MCA sample holders may contain hazardous samples (if such samples have been run by the user). The user should follow appropriate local and national regulations for their disposal.

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III. SOFTWARE INSTALLATION AND ACTIVATION

Your software

The software is supplied in a package as shown below. The package contains two USB compatible devices, one which contains the installers for the software along with some documentation. The other is a security dongle to prevent unauthorised copying or distribution of the software.

Each dongle is initially supplied in demo only mode and must be registered with Avacta Analytical before use. The software can be used for one month. If the software is not registered after this time then it will not be possible to use the software.

To register and activate the software contact Avacta Analytical directly by emailing optim@avacta.com supplying the serial number of your instrument, the date of installation, and who installed the instrument (either Avacta or one of our distribution partners).

Installing the Optim 1000 client software

It is only necessary to install the client software if you are upgrading from a previous software version. Your instrument will come supplied with the software pre-installed and you will only need to perform these steps if you have not obtained a controlling PC from Avacta Analytical, or if you reinstalling after a Microsoft® Windows® system failure.

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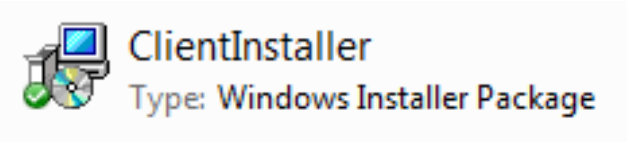
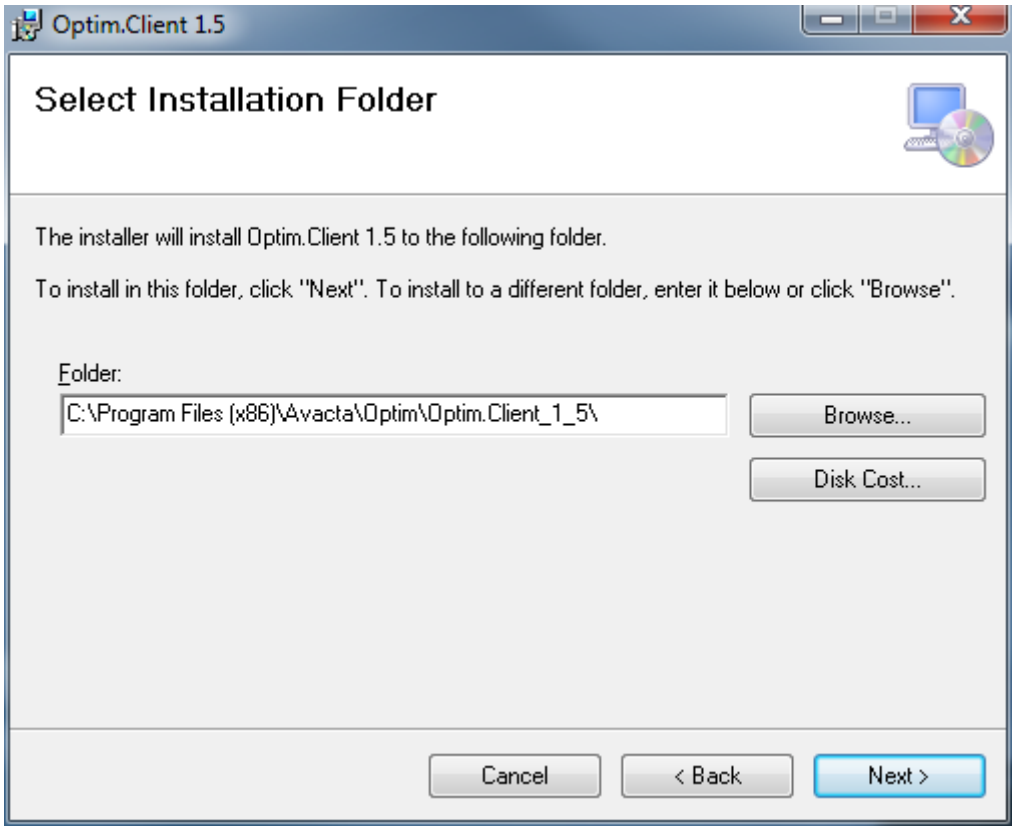
IMPORTANT - The Optim 1.1 database is not compatible with the v1.5 software. If you have upgraded your software it is not possible to load previously acquired data into the new version of the **client** software. As a result the run numbers will reset to 1. The old version of the software is still present on the system so this can be used as before but will not communicate with the instrument.

Insert the memory stick into the desktop PC that is attached to the instrument.

To install the Optim 1000 software v1.5 simply double click on the ClientInstaller.msi application. This will install the application onto the desktop PC that came with your instrument.



The installation software will then start to install the Optim client software (and if you are updating, a new updated database also) onto your computer. The software will prompt you for a location to install the software. Select the default location, or choose a location of your own by clicking "Browse…" before clicking "Next >".


Optim.Client 1.5
Select Installation Folder
The installer will install Optim.Client 1.5 to the following folder.
To install in this folder, click "Next". To install to a different folder, enter it below or click "Browse".
Folder:
C:\Program Files (x86)\Avacta\Optim\Optim.Client_1_5\
Browse...
Disk Cost...
Cancel
< Back
Next >

Once the software has been installed you will be prompted to configure the Optim Client 1.5. To do this, click "Next >" in the window below.

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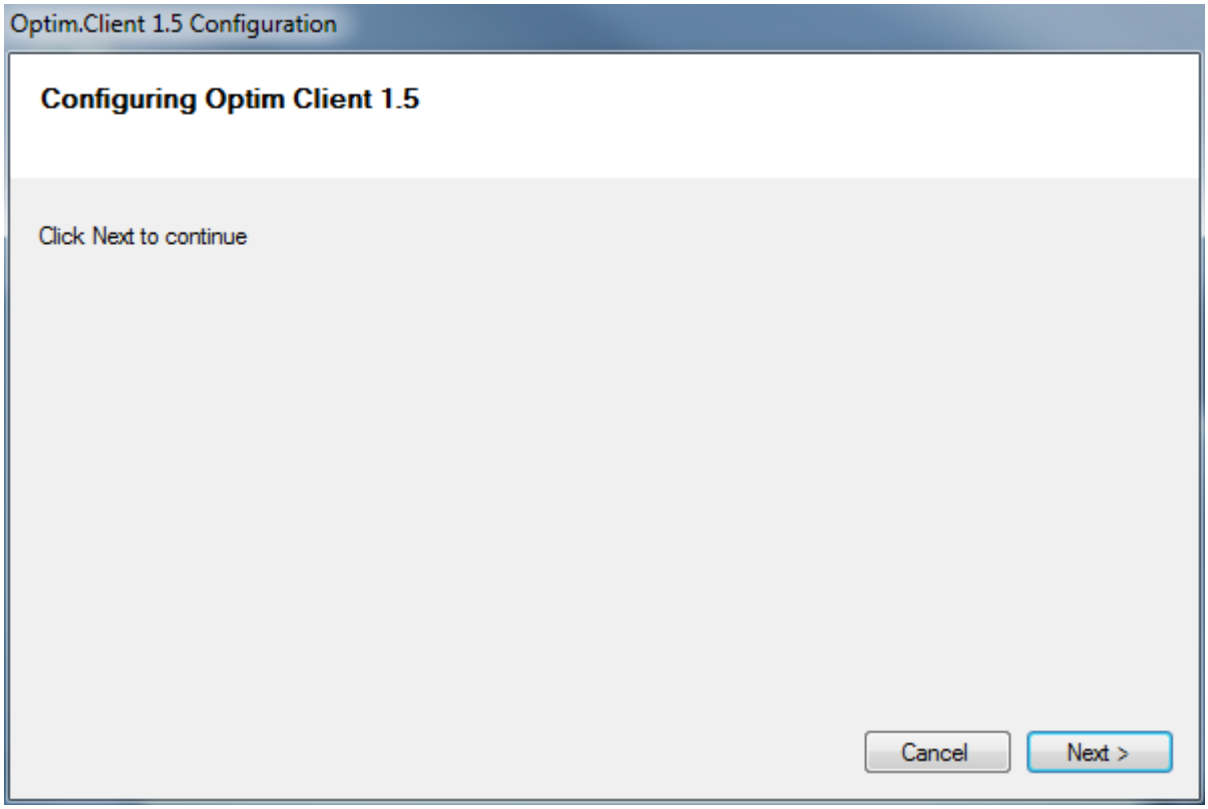
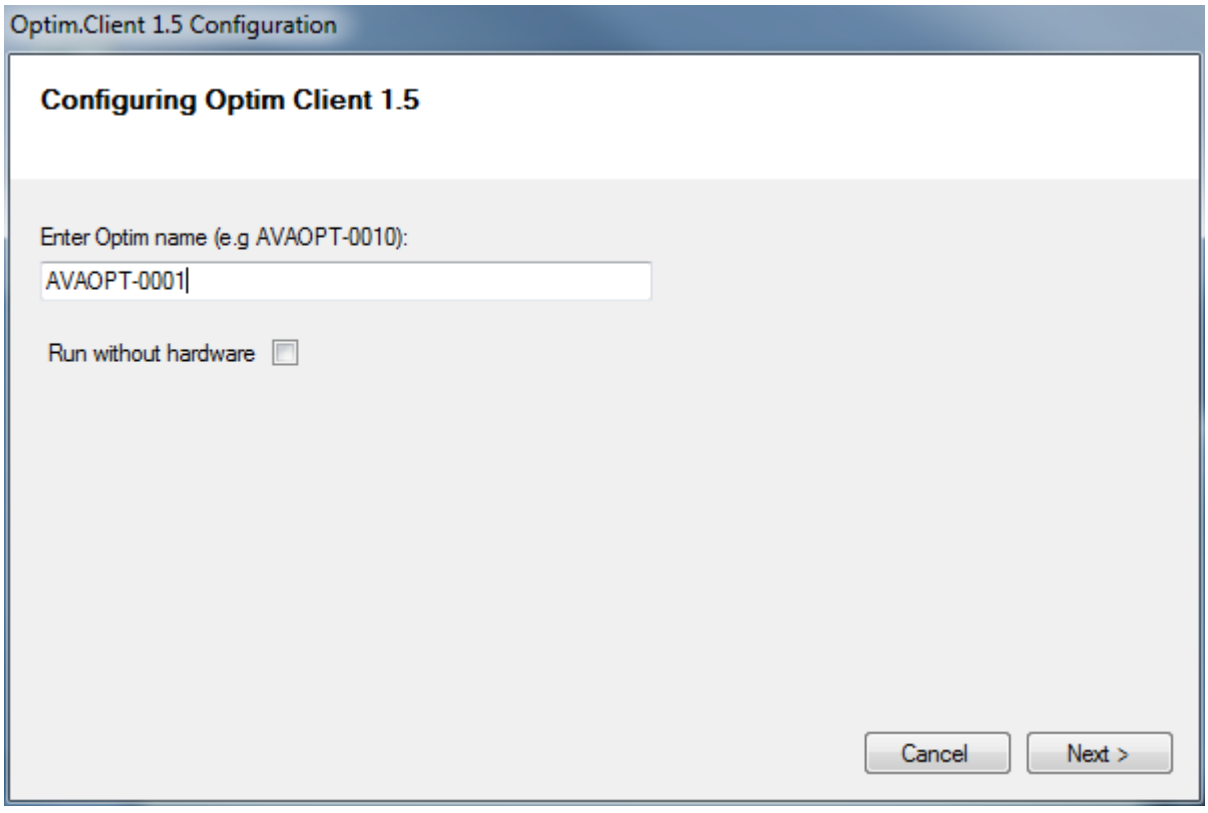


You will then need to enter the hostname of the instrument that you are connecting to. If you are installing the software on a PC supplied with an Optim 1000 instrument by Avacta Analytical then this should be automatically populated with a valid hostname. Otherwise, you will need to enter this here. The hostname you require will be AVAOPT-xxxx where xxxx are the last four digits of your instrument serial number.



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The installation software will then install the Optim Client 1.5 database. You will not need to interact with the installer during this time.

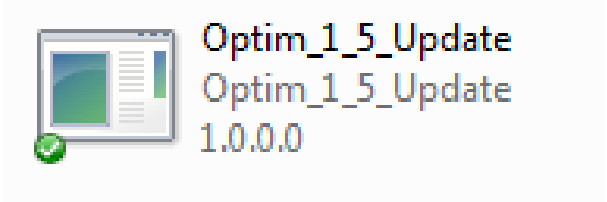
Once completed, a series of shortcuts will appear on your desktop. These shortcuts will help you use the Optim Client software efficiently.

- **Optim.Client 1.5** – this is the software itself. Double clicking on this will start the software and begin communication with the instrument
- **Edit formulation** – this will allow you to edit the formulation information
- **Optim Manual** – this is the user manual for v1.5 of the Optim instrument
- **Optim Quick Start Guide** – a simple and quick way to get started using Optim
- **Export 1.5** – where files exported from the client software are saved
- **Import 1.5** – the source of formulation information for importing

DO NOT USE THE FOLLOWING UNLESS DIRECTED TO BY AN AVACTA REPRESENTATIVE:

- **Optim Reset 1.5** – a tool used to restart sub components of the Optim instrument
- **Database Config Tool** – a tool to change the default parameters in the instrument

After this process has successfully completed and if you are upgrading from a previous software installation then double click on "Optim_1_5_update.exe" to update the Optim instrument you are connecting to.



Again, you will need to enter the target instrument that you wish to update.

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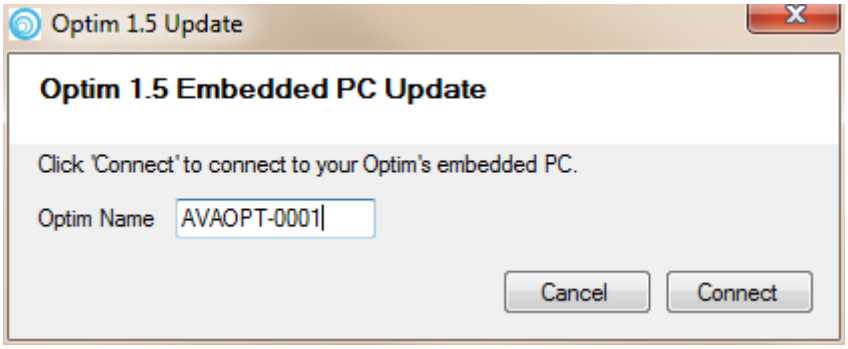
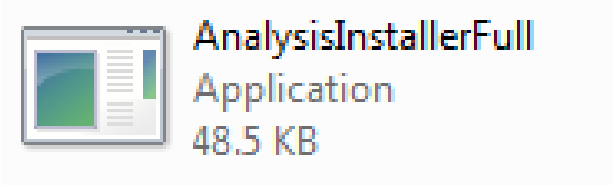


IMPORTANT - It is essential that the device installer is executed on the supplied desktop PC and that the Optim user is logged into the system at the time the installer is run, otherwise installation will fail due to insufficient permissions.

Installing the Optim Analysis software

Avacta Analytical has developed a new analysis tool, incorporating foundation technology called Igor Pro v 6.2 from Wavemetrics Inc. To install the Optim Analysis software v 1.5 simply extract the contents of the Optim Analysis zip from the memory stick, and double click on AnalysisInstallerFull.msi application.



This will install the subroutines, preferences files and the Igor Pro package onto your computer.

In order to use this software you must have a dongle for each computer on which you wish to use the software. You may install the software on one computer only, and any subsequent installations will require you to purchase an additional licence and dongle from Avacta Analytical.

The Optim Analysis software loads and saves data from defined folders on the installed computer. The default locations are configured such that raw data (in the form of spectra saved by the client software) is loaded from:

C:\Optim.Client\Database\OptimDB\Files\Capture

Data that is stored in studies are by default saved in a folder called "Optim Studies" that can be found in the users Documents folder, and accessed either directly or via a shortcut on the desktop.

If you are installing the analysis software on a computer that is not connected to an instrument and you wish to load data from a different location please refer to later sections which provide information on how to achieve this.

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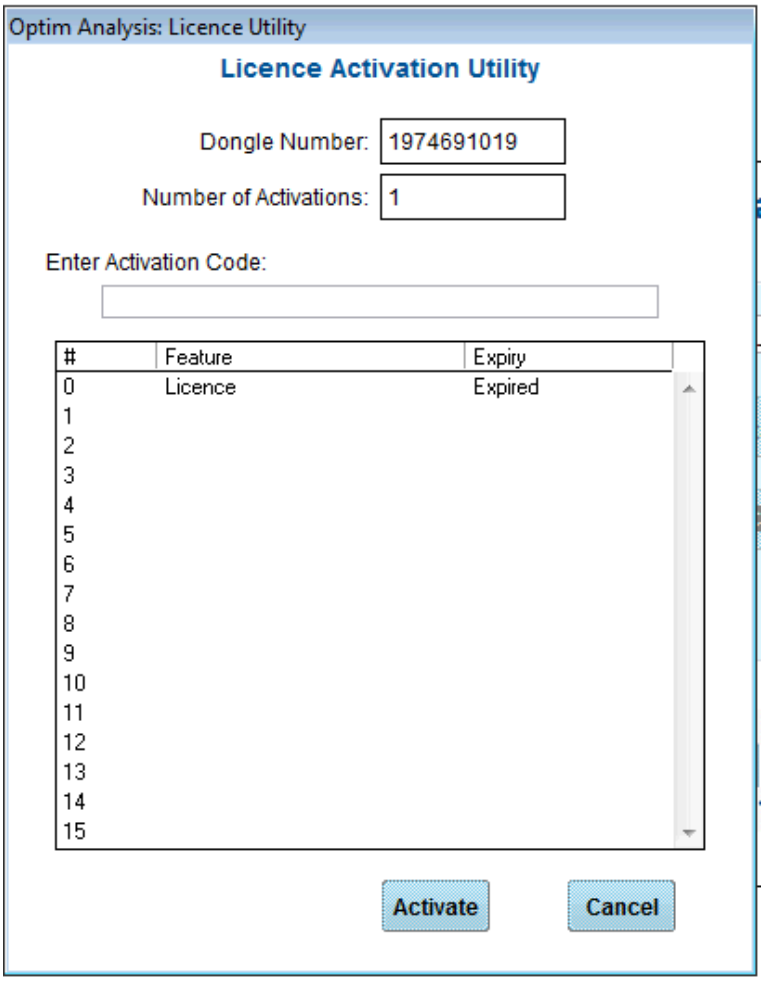
Activating the analysis software

The analysis software features a copy protection dongle that requires initial activation before analysis can be saved or exported.

To activate the software select the **OptimAnalysis** menu and the *Update Licence* option.



You should then contact Avacta Analytical Ltd *via* optim@avacta.com with your name, Optim serial number, the dongle number and the number of activations shown above. Avacta Analytical will send an activation code that will enable you to use the software without limitations while the dongle remains inserted.

If you remove the dongle you will not be able to save or export data from the software.

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THE CLIENT SOFTWARE AND INSTRUMENT HANDBOOK

The purpose of the Client software Handbook is to guide a user through the day-to-day operation of Optim 1000. "Starting a New Experiment" guides the Analyst user through every step of the settings and options that are involved in running a new experiment. Sections in the analysis segment of this chapter introduce the user to loading and processing data after it has been acquired. Some settings are hidden from the Analyst user and must be set up by the Administrator. For instructions on those features that are exclusive to the Administrator, and for in-depth descriptions of the pre-programmed software routines such as calibrations and analysis macros, read the later sections that appear later in this handbook.

The purpose of these later sections is to guide an Administrator-level user through the additional features of the software and to give further information about the default settings and options. Here you will find detail about the calibration functions applied to the raw data, the logic of the temperature control feature and some other useful information.

The administrator information is arranged into three sections: User Management, Data Management and Experiment Settings.

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IV. GETTING STARTED

This section contains all the information you need to get started with the Optim 1000 instrument and its accompanying software, including how to power the instrument on and off safely. The Optim software will already be installed on the PC.

Please note that the Optim Client software can only be supported on the PC connected to the Optim 1000 and should not be installed or copied onto any other computers.

In this part of the guide you will find information about the screen layout, the user access levels and an overview of how the software manages data. For quick reference, Section VII has a quick-start guide, which summarises the main steps involved in making a measurement with Optim 1000. There is also a flowchart to demonstrate the typical decisions taken by a user during the measurement process.

IMPORTANT - For safe operation of the instrument, the user is advised to follow the instructions and guidelines given in this document.

DANGER - The user should NOT remove any of the panels on the instrument. With the instrument turned on and the side panels removed, there is a significant risk of exposure to potentially harmful laser radiation, high voltage electricity and hot surfaces.

The service engineers may need to remove these panels whilst operating the instrument. They should assess the Risks associated with the Hazards, and take appropriate action to reduce these Risks to an acceptable level.

A set of risk assessments for the user are provided in the appendix of this document.

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V. POWERING OPTIM ON AND OFF

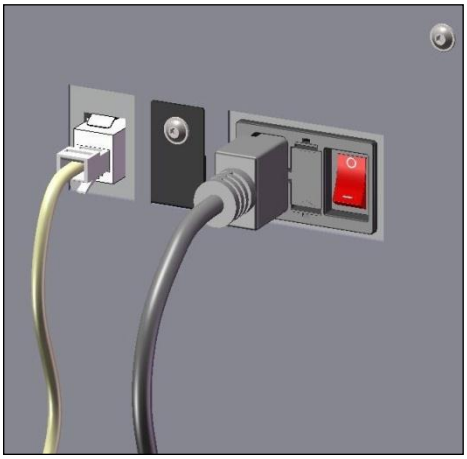
Turning On Optim

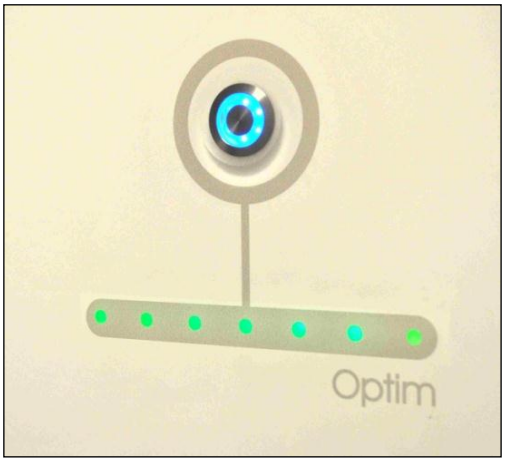
Optim 1000 has two power switches:

1) Firstly, at the top of the rear panel, where the power and data leads enter Optim, a rocker switch controls the overall power to the instrument.

   Switching this on will activate the power to the internal sub modules, including the laser sources.

> ⚠ **IMPORTANT -** Once this switch is set to On (1), all safety precautions should be observed.





2) The second power switch is located on the front of the instrument above the service status LEDs. Press this button once to start the Optim 1000 onboard computer.

The UV laser reaches optimal stability after one hour of warm up time.

Turning on the PC

The accompanying desktop personal computer is controlled independently of the Optim 1000 instrument. The computer can be used without the instrument being on, but not vice versa. Please refer to the PC manufacturer's manual for instructions on how to turn on the PC.

Automatic System Checks

When the Optim 1000 has been turned on, the service status LEDs (towards the top right of the front panel) will indicate the status of the constituent sub modules.

When Optim has been completely switched on and has booted up, six of the indicator LEDs on the front panel should become green. The central LED should

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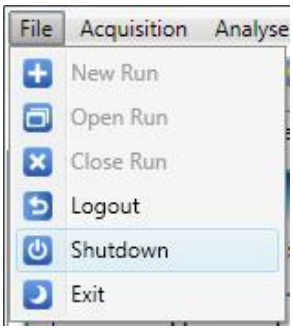
change to amber. IF ANY OF THE LEDS REMAIN RED, CONTACT YOUR SUPPORT AGENT.

Turning Optim Off

IMPORTANT It is advisable to switch off both the Optim 1000 and the accompanying PC when not in use.

1) Shut down the onboard computer by selecting *shutdown* from the **file** menu.



The blue light on the power switch (upper right of the front panel of the Optim 1000) should turn off after a few minutes.

2) Turn off the main power using the rocker switch near the back left of the instrument. The external PC may be used after Optim has been turned off.
3) When shutting down the software, always use **File** - *Exit*.

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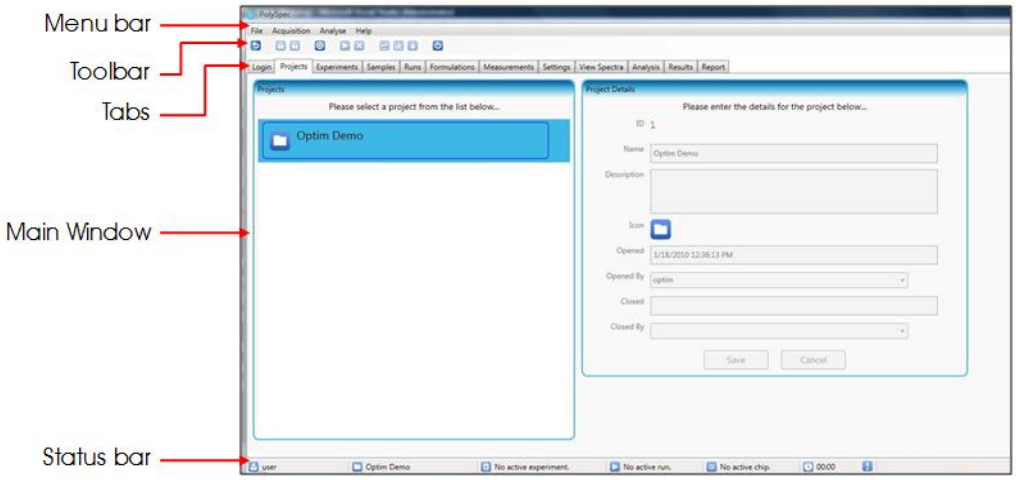
VI. INTRODUCTION TO THE OPTIM CLIENT SOFTWARE

Navigating the Software

> **IMPORTANT** Always switch the Optim 1000 fully on before starting the software.

The Optim 1000 client software controls the system during a measurement and then processes the measurement data through a series of calibration functions. A typical screen is shown below.



The **menu bar** gives access to the open/close Run, logout and shutdown features as well as providing a quick-look list of the acquisition and analysis functions. This manual can be found in *.PDF* format via a link in the **Help** menu.

The **toolbar** contains a selection of the most-used buttons to allow the user to quickly set up a measurement, collect data and process the results. These are summarised in the table below:

Symbol	Action
	Shutdown Optim.
	Log the user out of the Optim client program.
	Open the door to load samples into Optim.
	Close the door and return the stage to its start position.
	Acquire a single shot (this will not be saved to the database).

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Symbol	Action
	Start a Run (acquired spectra are saved to the database).
	Abort the Run (you will lose the current settings).

The **TIP** symbol in this guide indicates that a toolbar button or menu option can be used as a shortcut.

The **tabs** are arranged in roughly chronological order so that a user works from left to right when setting up a new experiment, taking a measurement, viewing the data and performing analysis. Hover over a tab heading for a description of the tab contents. Some buttons and features also have handy tooltip pop-ups to give you a snippet of useful information.

The **status** bar at the bottom of the window displays the following information:

- The user that is currently logged on
- The Project that is currently in use
- The selected Experiment
- The ID and title of the active Run
- The MCA configuration (number of wells in the current Run)
- Live status updates about the Optim 1000 instrument and a progress bar

File system

Database

The Optim software has an inbuilt **database** system that stores all newly acquired data sorted by Project, Experiment and Run. Within the software, data can be loaded by the Run ID and viewed, and all the results generated will automatically be saved to the database.

Exporting and Importing

Data can also be **exported** from the program. Any raw data, calibrated data or analysis results will be exported in the Excel format. Exported files will appear in a list in the Results tab and can be accessed in the folder *C:\Optim.Client\Export*.

> **IMPORTANT** It is advisable to save exported files immediately in a different file location as the *Export* folder will be cleared each time the software is restarted.

Formulation details for the MCAs can be **imported** from Excel into the database. The easiest way to do this is to use the "Edit Formulations" file that appears as a shortcut on the desktop to generate formulation information. It is also possible to import files from elsewhere on the hard drive by entering the file path details in the import window.

User Levels

There are three levels of user in the Optim software:

1. Analyst
2. Supervisor
3. Administrator

The **Analyst** user-level is a simplified user-interface that has been designed to enable an operator to use Optim, but restricts access to more advanced features. An Analyst user can prepare and load samples into Optim, take measurements, analyse results and generate reports.

A Project **Supervisor** has the same access level as an Analyst user except that they are able to create a new Project and manage the user access to that Project.

The **Administrator** user-interface allows access to the user management area and includes advanced Data management in addition to all of the functions that are available to the Analyst and Supervisor users.

User tab access in Optim software

The table below indicates which level of user can access each of the tabs in the current version of the Optim software.

User level / TAB	Analyst	Project Supervisor	Administrator
1) Login	✓	✓	✓
2) Users		✓*	✓
3) Companies			✓
4) Projects	✓*	✓	✓
5) Experiments	✓	✓	✓
6) Samples	✓	✓	✓
7) Runs	✓	✓	✓
8) Formulations	✓	✓	✓
9) Measurements	✓	✓	✓
10) View Spectra	✓	✓	✓

* User has limited access to the features of this tab

Structure Overview

The Administrator defines a user identity and assigns a list of projects to that user. Within a Project, the user must choose one of the experiments to run. There are four different types of experiment available. The experiments contain pre-programmed

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settings designed around characterising certain properties of samples for typical Optim applications.

The diagram below is a simplified view of the structure of the software. At first glance this may appear complex, but many of the processes illustrated here are default settings that the user need not be concerned with. Further detail on all of the stages depicted can be found further on in the manual if you stay awake to read that far.

A more experienced user can use the diagram to understand how settings and data are handled by the software.

Square-edge boxes represent processes that happen automatically, whereas round-edge boxes indicate that the user must interact with the software in some way. In this diagram, the term "user" includes all levels of user. Please refer to the Glossary at the end of this guide for other definitions.

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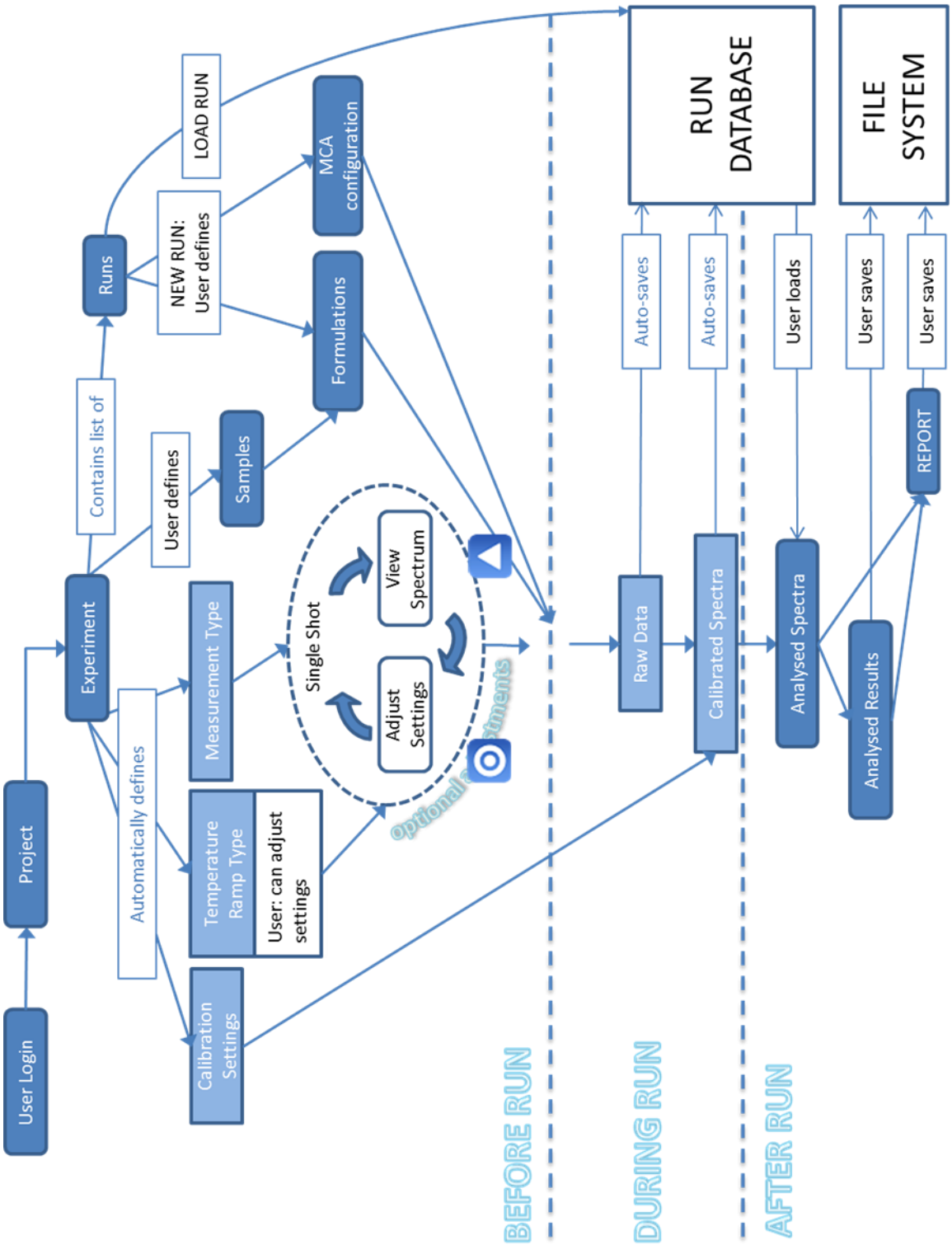
User Login
Project
Experiment
Contains list of
Runs
LOAD RUN
NEW RUN: User defines
User defines
Samples
Formulations
MCA configuration
Automatically defines
Measurement Type
Temperature Ramp Type
User: can adjust settings
Calibration Settings
Single Shot
View Spectrum
Adjust Settings
optional adjustments
RUN DATABASE
FILE SYSTEM
Auto-saves
Auto-saves
User loads
User saves
User saves
Raw Data
Calibrated Spectra
Analysed Spectra
Analysed Results
REPORT
BEFORE RUN
DURING RUN
AFTER RUN

VII. QUICK-START GUIDE

Quick-Start Outline

Here is a brief summary of how to obtain stability information about your molecule of interest using the Optim 1000.

- **Turn on** Optim, first by the switch at the back, then the button at the front. Switch on the PC.
- **Allow at least one hour for the laser in the instrument to warm up.**
- When all the lights at the front of Optim are no longer red, open the Optim Client 1.5 software and **Login** to a **Project**.
- Select an **Experiment** to start a new **Run.**
- Decide the **MCA configuration** (number of wells) you wish to use and input the **Formulation** details for your **Samples**.
- Fill the micro-cuvettes with the formulations. **Open the door**, pull out the tray and load the **Micro-Cuvette Arrays** (MCAs) onto the hot plate, starting from the left. Place the lid on the copper plate. Push the tray in and **Close the door**.
- View the **Measurement** details, amend the temperature step settings and start the **Run**.
- View the calibrated spectra in the **View Spectra** tab as they are acquired.
- When the Run is finished, go to the **Optim Analysis** software to begin analysing the data and load the run.
- Perform **Primary Analysis** to produce a set of unfolding curves and scatter data.
- Perform **Secondary Analysis** to find the equilibrium midpoint of the unfolding curves and the temperature of aggregation onset. Compare these results to find the more stable formulations for the molecule of interest.
- When finished, **Shutdown** Optim using the software. Wait until all the lights go red and the blue light goes off. Then it is safe to turn off the switch at the back.
- Use the menu option ***File → Exit*** to close the Optim Client Software. Save any exported files in a new folder. Shutdown the desktop PC.

If pictures are more your thing, overleaf is a flowchart which guides the Analyst user through this process. Simply follow the arrows and make a decision anytime you reach a blue diamond.

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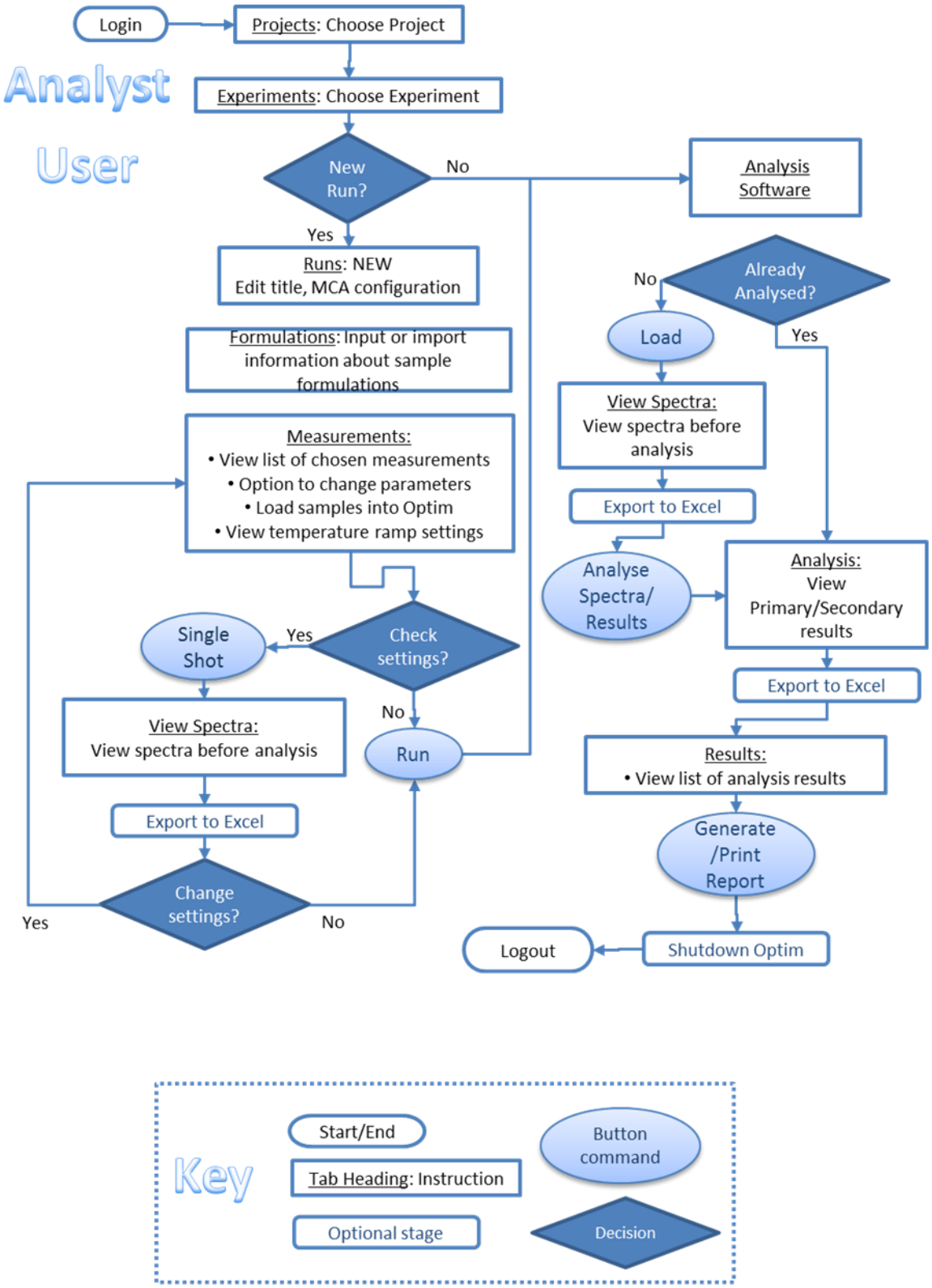

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VIII. STARTING A NEW EXPERIMENT

This part of the manual covers the basics of operating the instrument. It includes all details that are relevant to an Analyst-level user to step them through the process of loading samples into the Optim 1000 instrument, making a set of measurements, analysing the results and producing a report. The more challenging task of interpreting the results will be left to a more knowledgeable scientist-type person and will not be discussed in this manual.

Before an Analyst can start a new experiment, the Administrator must set up the new user's login details and create a Project in the database.

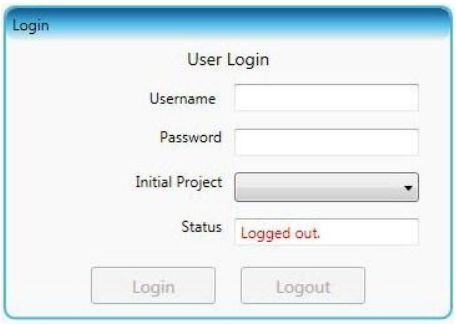
To Begin

Ensure that Optim is switched on:

- You should see a ring of blue LEDs and a row of green lights on the front of the instrument (one may remain amber until the software is started). If some or all of the lights are off, please refer to Section I for instructions on how to power up the instrument.
- Start up the Optim 1000 Client software by double clicking the link on the PC desktop – the **Login** tab should be displayed. The software cannot be run when the Optim instrument is switched off.

Login

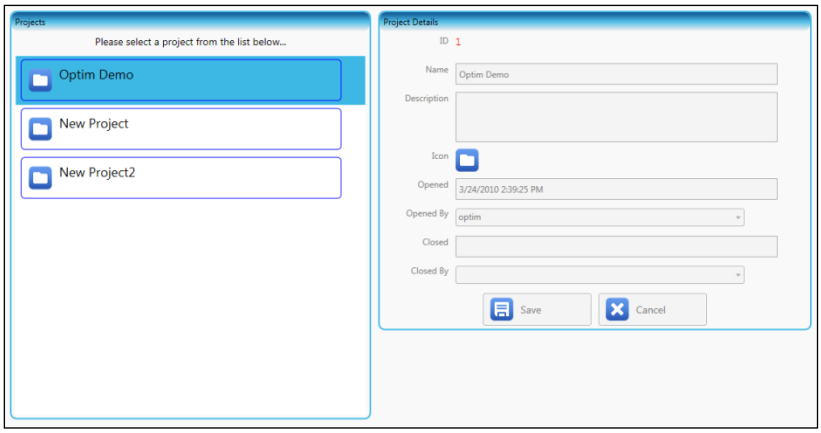
The Analyst's login details will be set up by the Administrator. The Administrator (or Project Supervisor) also has control over which projects are available to Analyst users.



- Insert your username and password.
- Choose a Project from the drop-down list.
- Click LOGIN.

Projects

To view the description of a Project or to change to another Project after you have logged in, use the **Projects** tab.



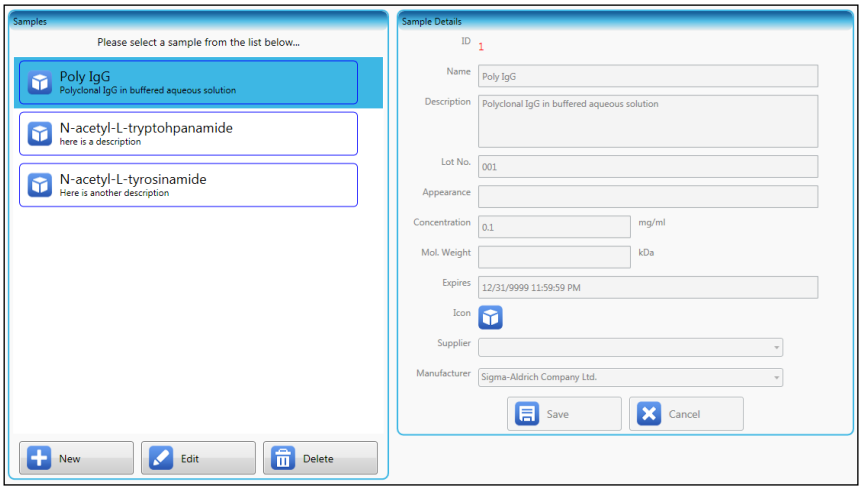
- In the list on the left, click on the Project you wish to work on.

All the settings and data for that Project will then be loaded.

If you have been assigned the responsibility of being a Project **Supervisor**, you can edit the details of the Project. You also have permission to create a new Project.

Samples

This tab contains information about the samples that will be investigated as part of the selected Project. Only samples relevant to the selected Project will be listed here. Any samples listed on this tab will also appear in a drop-down list in the **Formulations** tab.



To add a new Sample to a Project:

- Click NEW.
- In the window on the right, type in a sample *Name* (ID) and brief *Description*. These will appear in the list of Samples on this page and in the drop-down menu on the **Formulations** tab. [Note: The description field has a character limit and will be highlighted in red if the description is too short.]

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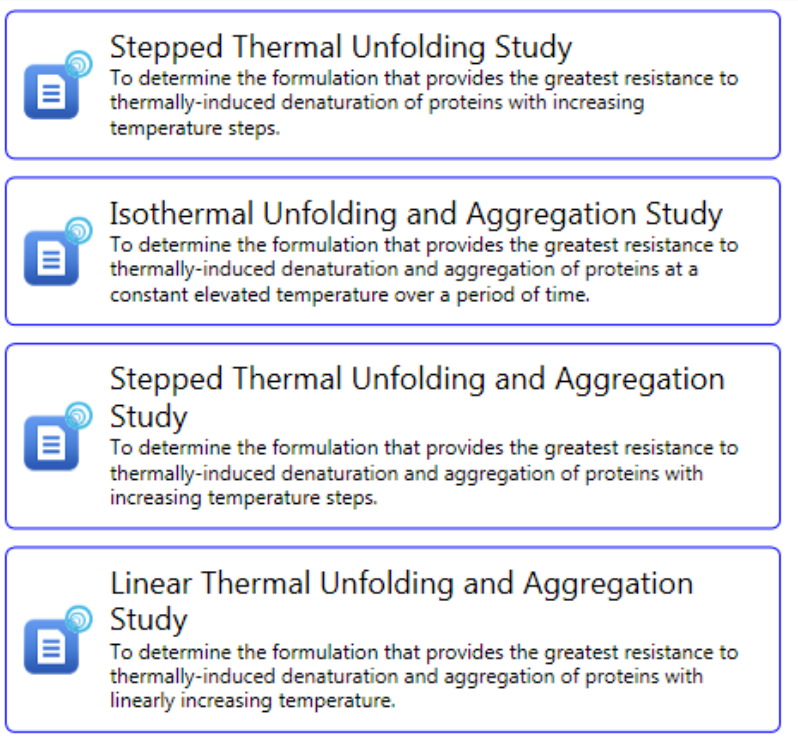
- All other information is optional. You can record the *Lot No., Concentration, Appearance, Molecular Weight* and *Expiry date* in this window.
- If your sample has been supplied or manufactured by another company, select the appropriate companies from the drop-down lists at the bottom of the window (the list of companies will be managed by the Administrator).
- Click SAVE.

Any Samples associated with a given Project will be listed by *Name* in the **Formulations** tab drop-down list, which will be discussed later.

Experiments

The user is provided with four templates for experiments (listed below). Within a Project, any or all of the experiments can be performed. Each of the templates has predefined settings that are suitable for typical Optim applications. These are described in further detail in the **Method** box on this tab and in Section XI on page 59 of this handbook.

- Choose an Experiment template by clicking once on one of the four options:


Stepped Thermal Unfolding Study
To determine the formulation that provides the greatest resistance to thermally-induced denaturation of proteins with increasing temperature steps.
Isothermal Unfolding and Aggregation Study
To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins at a constant elevated temperature over a period of time.
Stepped Thermal Unfolding and Aggregation Study
To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins with increasing temperature steps.
Linear Thermal Unfolding and Aggregation Study
To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins with linearly increasing temperature.

- In the pop-up box:
  - Click NEW to start a new Run,
  - Or, to view historical data, choose a Run from the drop down list and click LOAD.

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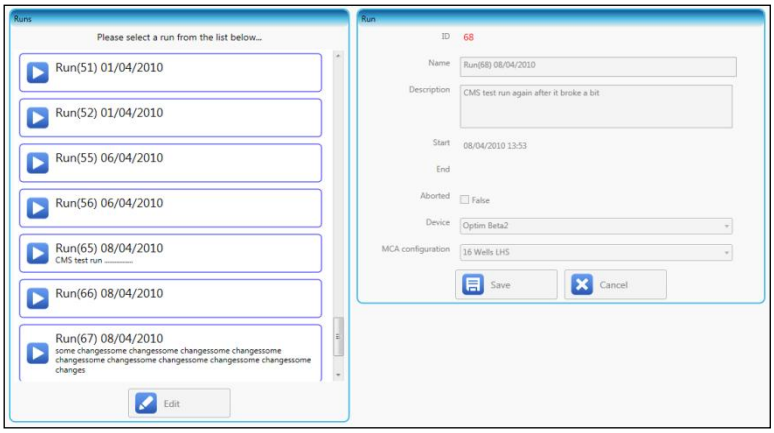
The Optim software will now load the settings for the chosen Experiment.

Runs

This tab shows a list of all completed runs for the chosen Experiment in the selected Project. Click on a Run to view the details in the box on the right of the screen. If you are beginning a new Run, it will appear at the bottom of the list.



- If the most recent Run is not already highlighted, click on it and click EDIT to edit the title and description of the Run you are about to start.
- Note down the Run ID highlighted in red at the top of the **Run Details** window. This ID can be used to find the Run again quickly using the *Open Run* command in the **File** menu, and will be used in the data analysis.
- Choose the *MCA configuration* (the number of formulations in the Run) using the drop-down list. The *Device* selection should show the default Optim version.

IMPORTANT It is important to select the correct MCA volume here as each behaves differently during an experiment. 1 µL MCAs have an initial optimisation step to locate the cuvette centre but this isn't necessary in 9 µL MCAS. Similarly each has a slightly different thermal response so are calibrated differently.

- Click SAVE.

When you have filled in the Run details, go to the **Formulations** tab to fill in the data about the contents of the micro-cuvettes.

Formulations

This tab is designed to store all the information about the contents of the micro-cuvettes in the current Run. Data can either be input directly to the database, or imported from Microsoft Excel. The Excel file must be in the correct format to be compatible with the Optim software.

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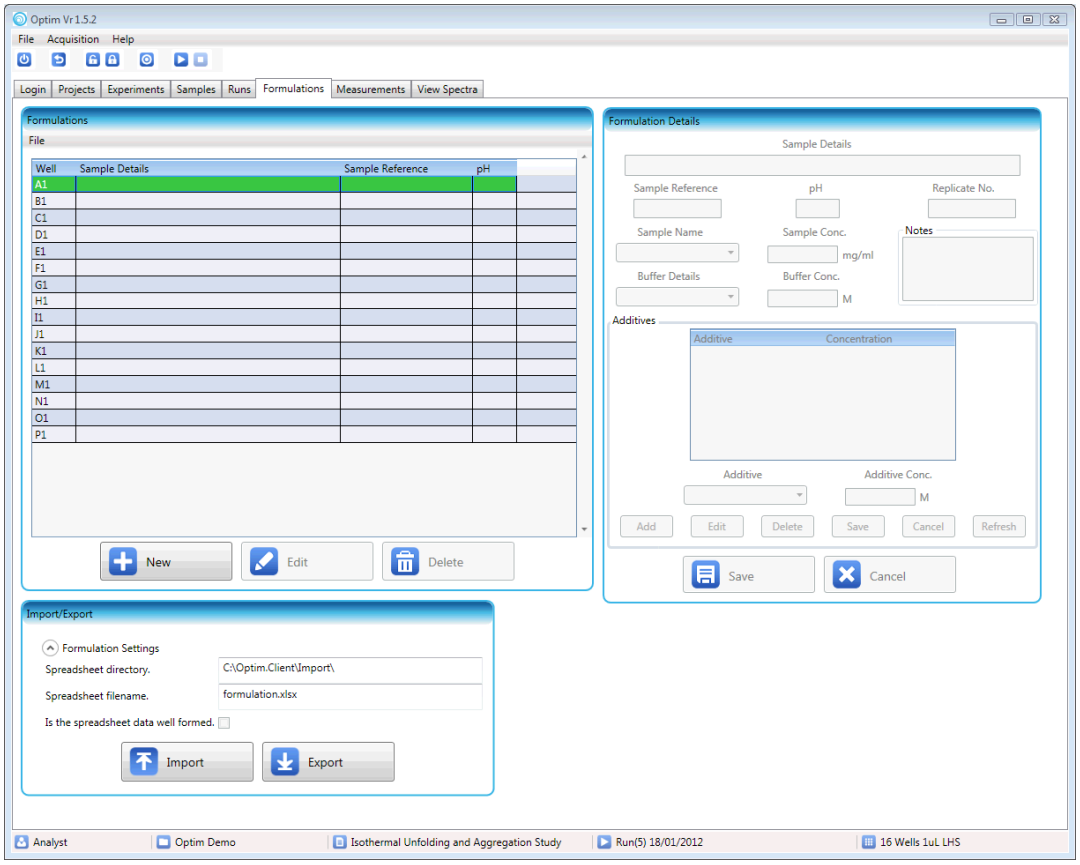
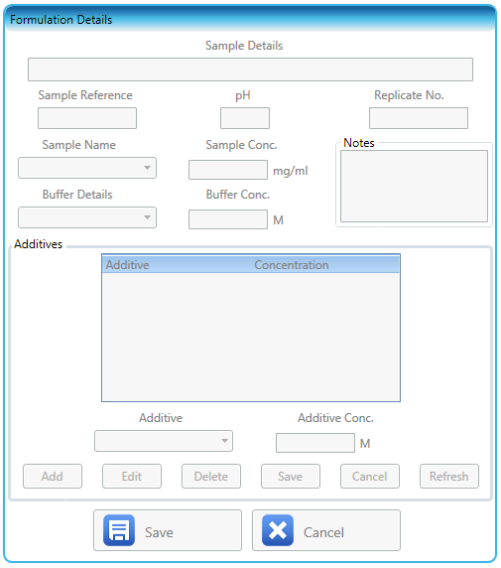
You should see an empty table with the same number of rows as there are wells in the MCA configuration you have chosen.


Optim Vr1.5.2
File Acquisition Help
Login Projects Experiments Samples Runs Formulations Measurements View Spectra
Formulations
File
Well Sample Details Sample Reference pH
A1 B1 C1 D1 E1 F1 G1 H1 I1 J1 K1 L1 M1 N1 O1 P1
New Edit Delete
Formulation Details
Sample Details
Sample Reference pH Replicate No.
Sample Name Sample Conc. mg/ml
Buffer Details Buffer Conc. M
Notes
Additives
Additive Concentration
Additive Additive Conc. M
Add Edit Delete Save Cancel Refresh
Save Cancel
Import/Export
Formulation Settings
Spreadsheet directory. C:\Optim.Client\Import\
Spreadsheet filename. formulation.xlsx
Is the spreadsheet data well formed.
Import Export
Analyst Optim Demo Isothermal Unfolding and Aggregation Study Run(5) 18/01/2012 16 Wells 1uL LHS

To <u>input</u> formulation data to the database manually:

- In the **Formulations** box, highlight the well you wish to edit and click NEW. (If you wish to change data that has already been entered, click EDIT).
- In the **Formulation Details** box, fill in the following information for each well that contains a sample.



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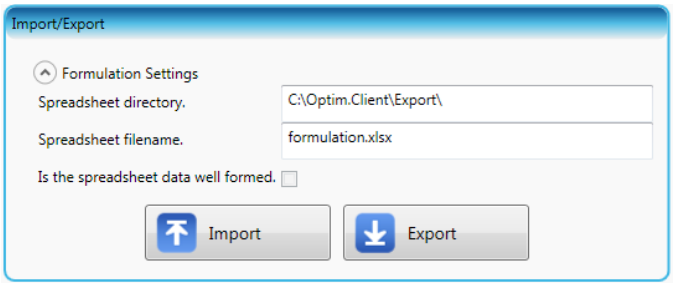
- Standard Data:
  - **Well Sample Name:** Write a brief description of the formulation in the selected well. This will be displayed in the list of acquired spectra and results.
  - **Well Sample Ref.:** This reference is for the user only and should be chosen according to the user's company policy.
  - **Sample:** Select a sample molecule from the drop-down list and enter the concentration (in mg/ml).
  - **Buffer:** Select a buffer from the drop-down list and enter the concentration (Molar, M). Administrators can add to the list of buffers via the **Samples** tab. For instructions, see section X on page 55.

- Optional Data:
  - **pH:** Type in the pH for this formulation.
  - **Replicate No.:** Keep a record of duplicate formulations by labelling them with the same replicate number.
  - **Additives:** To add an additive to the formulation, click NEW, select an additive from the drop-down list and type in the concentration (Molar, M). Click SAVE. The additive will now be listed (Highlight an additive in the list and use the buttons below to EDIT or DELETE it as required). Administrators can add to the list of additives via the **Samples** tab. For instructions, see section X on page 55.
- When you have entered all the required information, click SAVE.
- If you wish to use this list of formulations again, there is an option to EXPORT the data to an Excel file (located in *C:\Optim.Client\Export*).

To import formulations you have previously edited and exported:

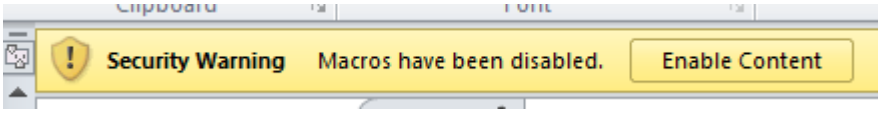
- Note the filename that you used to EXPORT the data to an Excel file (located in *C:\Optim.Client\Export*).
- Change the path in the spread sheet directory to reflect where you exported the formulation too before.



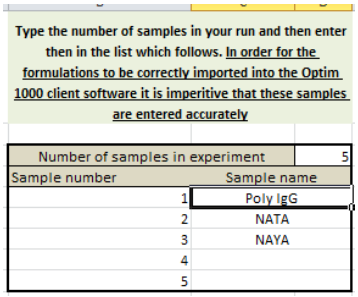
- Click IMPORT.

To import formulations from Microsoft Excel:

- Open the shortcut "**Edit Formulation**" on the desktop to open the Excel tool.
- If you see a Security Warning at the top of the window in Microsoft Excel, click Enable Content before continuing.



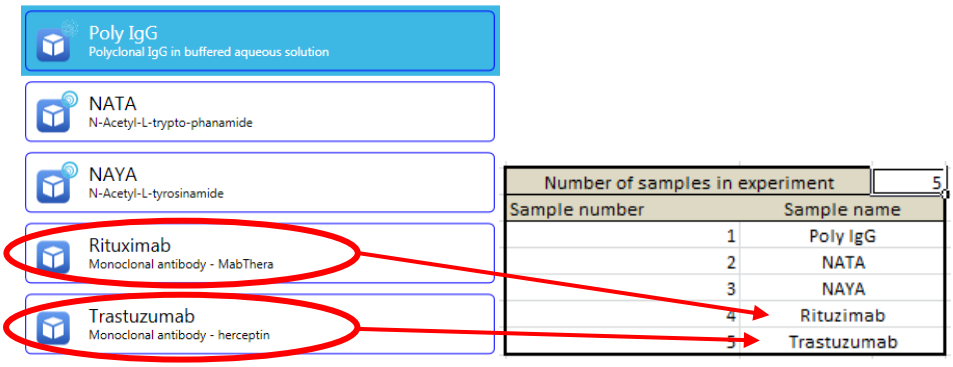
- You will be presented with an Excel sheet called "Project samples". Change the number of samples from 3 to match the number of samples you have on the Samples tab in the client software.



Type the number of samples in your run and then enter then in the list which follows. In order for the formulations to be correctly imported into the Optim 1000 client software it is imperitive that these samples are entered accurately

Number of samples in experiment	5
Sample number	Sample name
1	Poly IgG
2	NATA
3	NAYA
4	
5	

- Add any new samples to the **Sample** tab
- Note the example contained here has a deliberate mistake in the miss-naming of Rituximab in Excel template formulation editor. If such an error occurs in practice the user will be presented with an "Error no sample record named 'Rituzimab' found for well no. 1" warning. **(Please note: the Sample Name is case sensitive and must exactly match the name in the database.)**



- Proceed onto the Create Formulation sheet.
- Choose the correct *MCA configuration* and the *Number of additives* from the dropdown boxes at the top of the sheet – the sheet should expand or contract to show the relevant cells for editing. *[Note, due to limitations imposed by Microsoft, when using the Excel import template it is possible to enter up to three additives per sample well. If you wish to use more you must add them using the manual method outlined above.]*
- For each sample, select an entry for the Sample Name using the dropdown box at the side of each cell, and complete the rest of the formulation information.
- If you wish to perform a buffer background measurement (for example if you have fluorescing compounds in your buffer) the analysis software

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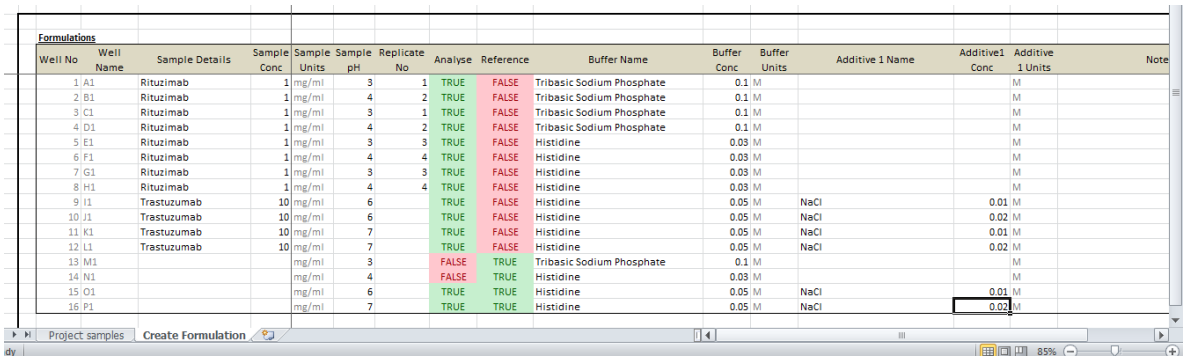
will attempt to find the spectra for the relevant buffer and subtract it from the sample spectra automatically. To do this:

- Enter identical formulation information in the well in which the buffer will be loaded, but leave the Sample Details column empty.
- Change the reference column entry to TRUE.
- Changing the Analyse column entry to FALSE will not affect the buffer subtraction, but the buffer spectra will not be analysed. It will not be possible to see how the buffer fluorescence and light scattering signals change with temperature.

- If you are measuring replicates, insert a value into the Replicate No column, all rows with the same replicate number will be grouped together as replicates in the Optim Analysis software.
- Type in a filename and click CREATE FORMULATION. A file will be created in the **Import** folder.
- In the software, go to the **Import/Export** box and input the Excel file name and location.
- Click IMPORT.

An example formulation editor sheet is shown below.



Formulations

Well No	Well Name	Sample Details	Sample Conc	Sample Units	Sample pH	Replicate No	Analyse	Reference	Buffer Name	Buffer Conc	Buffer Units	Additive 1 Name	Additive1 Conc	Additive 1 Units	Note
1	A1	Rituzimab	1	mg/ml	3	1	TRUE	FALSE	Tribasic Sodium Phosphate	0.1	M			M	
2	B1	Rituzimab	1	mg/ml	4	2	TRUE	FALSE	Tribasic Sodium Phosphate	0.1	M			M	
3	C1	Rituzimab	1	mg/ml	3	1	TRUE	FALSE	Tribasic Sodium Phosphate	0.1	M			M	
4	D1	Rituzimab	1	mg/ml	4	2	TRUE	FALSE	Tribasic Sodium Phosphate	0.1	M			M	
5	E1	Rituzimab	1	mg/ml	3	3	TRUE	FALSE	Histidine	0.03	M			M	
6	F1	Rituzimab	1	mg/ml	4	4	TRUE	FALSE	Histidine	0.03	M			M	
7	G1	Rituzimab	1	mg/ml	3	3	TRUE	FALSE	Histidine	0.03	M			M	
8	H1	Rituzimab	1	mg/ml	4	4	TRUE	FALSE	Histidine	0.03	M			M	
9	I1	Trastuzumab	10	mg/ml	6		TRUE	FALSE	Histidine	0.05	M	NaCl	0.01	M	
10	J1	Trastuzumab	10	mg/ml	6		TRUE	FALSE	Histidine	0.05	M	NaCl	0.02	M	
11	K1	Trastuzumab	10	mg/ml	7		TRUE	FALSE	Histidine	0.05	M	NaCl	0.01	M	
12	L1	Trastuzumab	10	mg/ml	7		TRUE	FALSE	Histidine	0.05	M	NaCl	0.02	M	
13	M1			mg/ml	3		FALSE	TRUE	Tribasic Sodium Phosphate	0.1	M			M	
14	N1			mg/ml	4		FALSE	TRUE	Histidine	0.03	M			M	
15	O1			mg/ml	6		TRUE	TRUE	Histidine	0.05	M	NaCl	0.01	M	
16	P1			mg/ml	7		TRUE	TRUE	Histidine	0.05	M	NaCl	0.02	M	

Project samples | Create Formulation

In this case, there are two sample molecules, Rituzimab [sic] and Trastuzumab. The following points are worth noting about this formulation:

1) There are four duplicates (the first eight formulations)
   a. A1 and C1
   b. B1 and D1
   c. E1 and G1
   d. F1 and H1
2) There are four buffers being measured
   a. M1 is the buffer for A1 and C1, and at each temperature the spectrum of M1 will be subtracted from that of A1 before analysis. The spectra of M1 will not be analysed
   b. N1 is the buffer of F1 and H1, with a comparable outcome as above
   c. O1 is the buffer for I1, and at each temperature the spectrum of O1 will be subtracted from that of I1 before analysis. The spectra of O1 will be analysed

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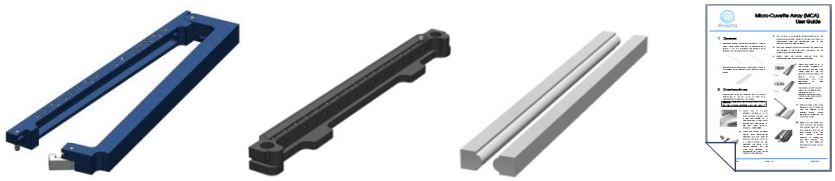
d. P1 is the buffer for L1

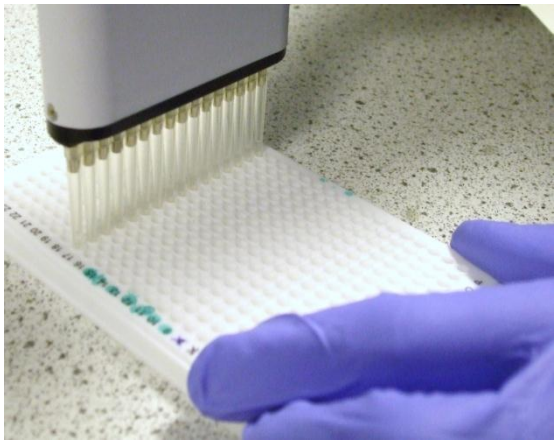
Now would be a good time to fill the micro-cuvettes with the sample formulations and check them against the list displayed on your screen.

The next two sections will describe how to prepare and load the micro-cuvette arrays (MCAs).

Preparing the Micro-Cuvette Array (MCA)

For making measurements with the Optim 1000, the user can purchase a quantity of Micro-Cuvette Array sample holders (MCAs). Preparation of samples for use in Optim is not covered in this document. For further information about how to handle the sample holders, please read the MCA User Guide.





Warning! - Take care not to touch the surface of the quartz cuvettes as this could significantly degrade the quality of the data.

1) Either use a single-channel pipette or pre-load the formulations into a row (e.g. A9-P9) of a microtitre plate, such as a conical well, low volume, 384 well plate (image: Starlabs E1042-3849).

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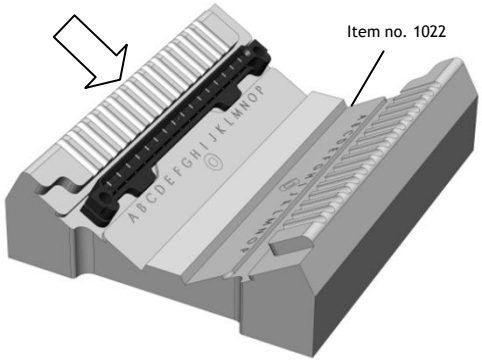
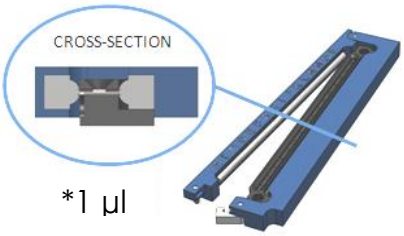
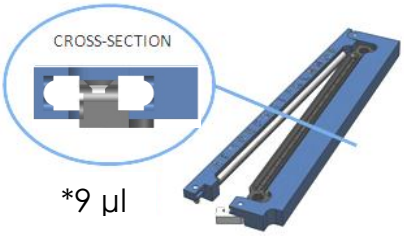
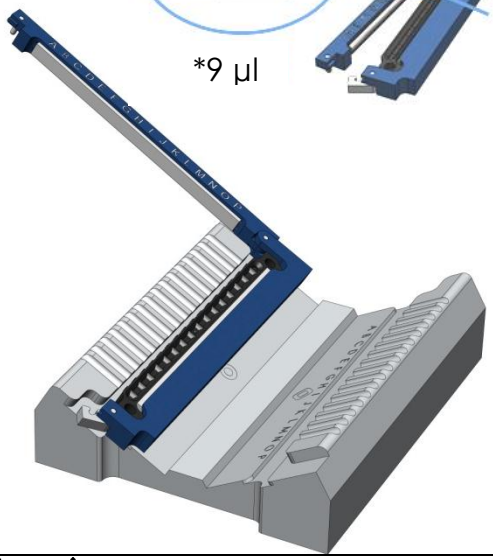
2) Place the black cuvette holder with pre-loaded cuvettes into the groove on the Filling Tool, so that it is held in place by the magnets.
3) Use a single or 16-channel micropipette, with an appropriate volume range, to collect the sample formulations from the pre-loaded row in the microtitre plate or sample vessel.





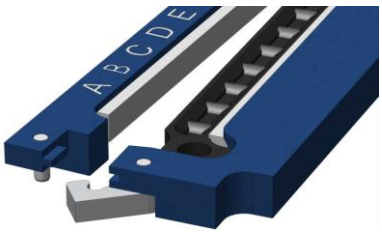




4) Align the pipette tips with the micro-cuvettes using the grooves in the Filling Tool, indicated by an arrow in the illustration.
5) Gently press the plunger, ensuring that the formulations flow into the micro-cuvettes.
6) Place one seal, in the correct orientation*, into each side of the blue frame. These will seal the ends of the cuvettes and ensure that the formulations do not evaporate at high temperatures.

*The round side of the seal should be in contact with the ends of the 1 µl cuvettes. The square side is used to seal the ends of the 9 µl cuvettes.

7) Slide the frame onto the cuvette holder, ensuring that the tongue and groove fittings mate correctly at each end of the black holder.
8) Gently lift the MCA off the Filling tool and close the hinged part of the cap, mating the tongue and groove near the clip. Apply gentle pressure to compress the seals against the ends of the cuvettes and secure the cap with the clip.



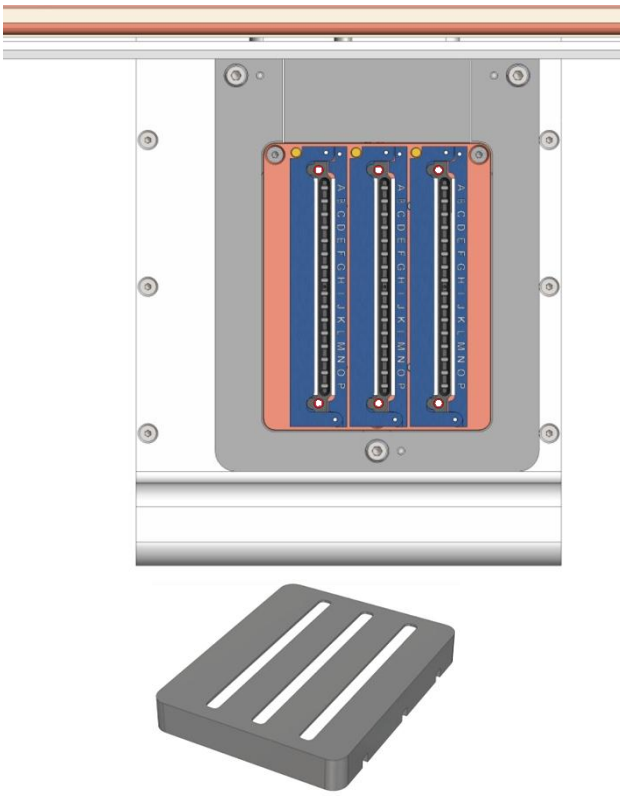
WARNING – Biohazard/Chemical Hazard. Your sample formulations may contain components that have potential to transmit infectious diseases or are dangerous chemicals. Follow all appropriate local and national regulations. Wear appropriate protective eyewear, gloves and clothing when handling such samples.

Loading the MCAs into Optim

- Open the **Measurements** tab. In the **Stage Control** window, click OPEN DOOR to access the sample drawer. This will open the door and

move the stage into a position where it is easily accessible from the door.

> **TIP** – Alternatively, click the OPEN button on the Toolbar.



1) Wait for the door to open fully and then pull the drawer out to its full extent. The LED will flash intermittently red and green.
2) Place the MCA sample holders onto the hot plate sequentially from left to right, ensuring that **each is secured by the brass spigots** and that **each is flat with no gap between the MCA base and the copper**.
3) **Place the insulating lid over the MCAs**.
4) Push the drawer all the way back in and check that the door LED flashes green.

- In the **Stage Control** window, click CLOSE DOOR to close and lock the door and return the stage to its default position.

> **TIP** – Alternatively, click the CLOSE button on the Toolbar.

Taking a Measurement

The chosen Experiment will have default settings for the measurements that the instrument will perform (for example, UV fluorescence). There is no need to change any settings at this stage, as the defaults have been chosen to be suitable for most applications.

Measurements

View the **Measurements** tab to see which measurements will be performed.

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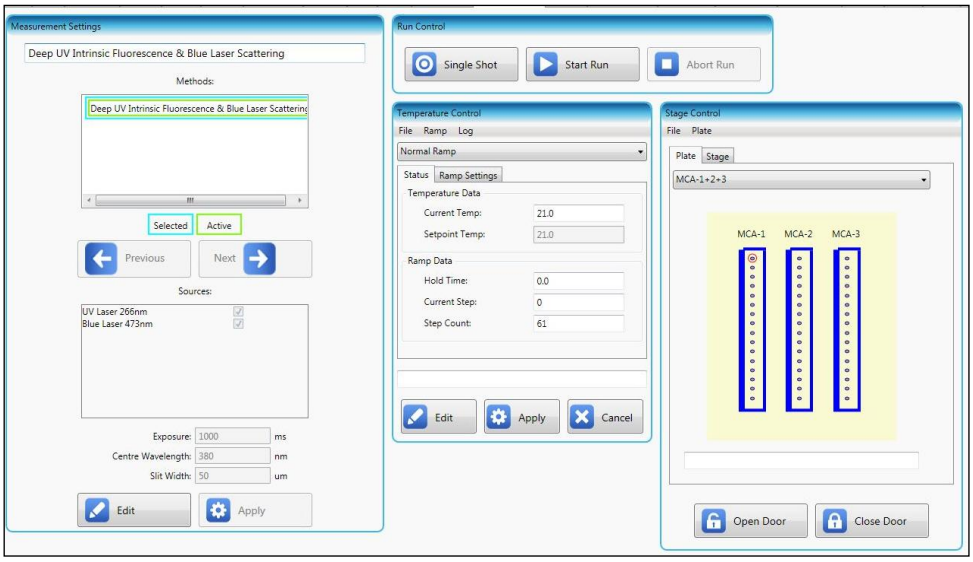

If you wish to change the default exposure time, slit width or centre wavelength:

- In the **Measurement Settings** window, click EDIT.
- Type in the required exposure time, slit width and centre wavelength.
- Click APPLY to apply the changes. It could take several seconds to apply the changes.

Temperature Control

The **Temperature Control** window displays the temperature settings that have been chosen for this Experiment:

Status: This tab displays the temperature, hold time and step count status throughout a Run.

Ramp Settings: For a "thermal ramp" type Experiment, the temperature will start at the *Start Temperature* and a measurement will be taken for each well of the MCA configuration. The temperature will then increase by the chosen increment size (indicated by *Temp Step)*. When the set temperature of the copper plate has been reached, the instrument waits for the designated *Temp Hold Time* and then takes another set of measurements. Measurements are taken at each temperature step until the *Stop Temperature* has been reached.

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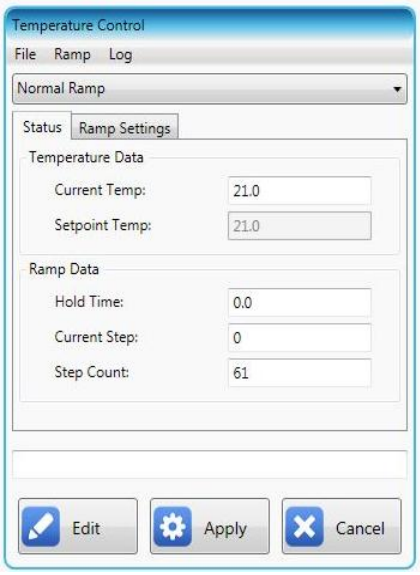

To alter the Ramp settings:

- Click EDIT.
- Type in a *Start Temperature* and *Stop Temperature* within the range from 15 °C to 100 °C.
- Type in the required *Temperature Step* Size (°C) and *Hold Time* (seconds).
- Click APPLY to apply the changes.

Isothermal Settings: For an "isothermal" type Experiment, the temperature remains constant within a tolerance of less than half a degree over the full range of operating temperature. Measurements are taken at each well and then the instrument waits for the designated *Wait Period* before beginning a second set of measurements. This process is repeated for the number of times indicated by the *Repeat Count*.

To alter the Isothermal settings:

- In the **Isothermal settings** tab, click EDIT.
- Type in the required *Temperature* (between 15 °C and 100 °C), *Wait Period* (seconds between measurements) and *Repeat Count* number.
- Click APPLY to apply the changes.

Linear Ramp: For a thermal unfolding using a linear heating ramp profile type Experiment, the temperature increases from a *Start* Temp to a *Stop* Temp at a constant rate that is defined by the user by *Rate*. All the samples are heated in the same way and at each point in time are at the same temperature. Measurements are taken at each well sequentially. After each spectrum is acquired, before moving to the next well the system waits for a period of time given by *Well Hold Time*. This

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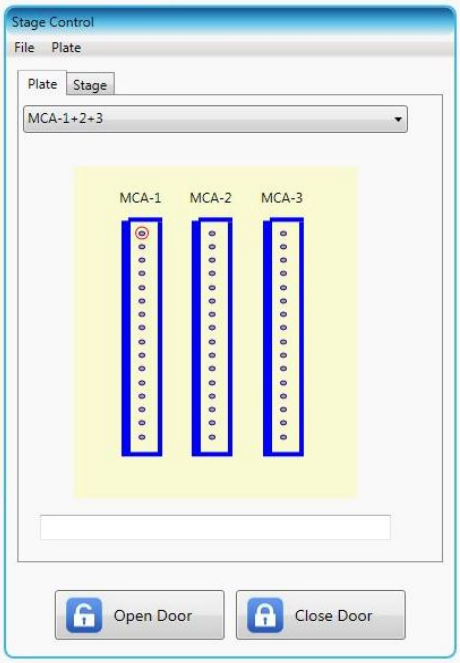
value can have a minimum value of 15 seconds, as any shorter value can lead to a break down in the linearity of the heating rate.

To alter the Linear Ramp settings:

- In the **Linear Ramp** tab, click EDIT.
- Type in the required *Start Temperature* and *Stop Temperature* (between 15 °C and 100 °C),
- Type in the required *Well Hold Time* (seconds between measurements of adjacent wells) and *Rate*.
- Click APPLY to apply the changes.

Stage Control

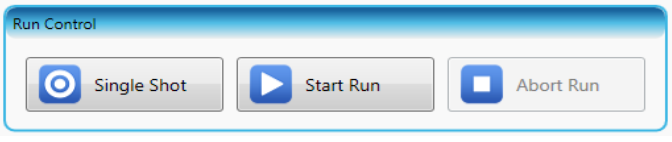
The **Stage Control** window displays the MCA configuration that has been chosen for the Experiment, which should be the same as the MCAs you have loaded onto the stage. During a Run, the stage moves the focus from well to well and measurements are taken at each one. As each well is measured, it will be highlighted in this window.



TIP – **NEW FEATURE –** clicking on a well will move the instrument optics such that the selected well becomes the active well. A single shot will now capture the selected well.

Run Control

The Run control window contains the buttons to take a single shot, start a Run or abort a Run. If a Run is aborted before completion, the partial set of data will still be saved to the database. **If you wish to restart the Run, you must opt to start a New Run from the Experiment menu and re-enter all of the settings as required**. (The Run settings have been recorded in the *.xml* file which can be accessed by clicking on the blue ID of a Run in the **View Spectra** tab.)



When you have checked or changed the measurement settings:

- You can verify your settings by taking a single snapshot (of each well) to preview the spectrum. Click SINGLE SHOT in the **Run Control** window.

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TIP – Alternatively, select *Single Shot* from the **Acquisition** menu or use the toolbar button.

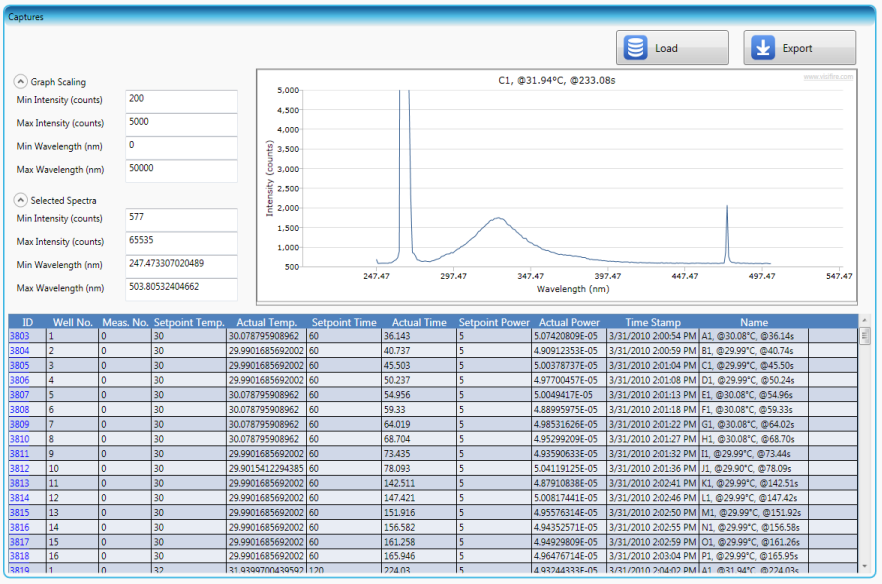
- The acquired spectrum will be immediately visible in the **View Spectra** tab, but will not be saved to the database. If you wish to save a copy of this spectrum, you can export the data (calibrated or raw) to Excel using the EXPORT button or by right-clicking the spectrum in the list.
- To begin a run, click START RUN in the **Run Control** window.

All spectra collected during a run will be saved to the database automatically and can be accessed at any time by selecting the appropriate Project → Experiment → Run.

TIP – Alternatively, select *Start Run* from the **Acquisition** menu or use the toolbar button.

View Spectra

In this tab, the user can view any of the spectra that have been acquired in the selected Run. These spectra have already been calibrated using the default calibration procedures. To view details of the calibration procedures see later in this manual.



If you have loaded a Run and there are no spectra listed in the **View Spectra** tab, click LOAD in this window and wait a few seconds for the list to be populated.

- Click on any spectrum to view it as a graph or

- Tick the *Auto Show Spectra* box to view the most recently acquired spectrum.
- Any spectrum where the detector has been saturated by an intensity that is too high will be displayed in red
- Change the view settings by typing values into the *min* and *max* boxes to the left of the graph, then selecting a different field.
- Sort the data by clicking on a column heading. Hold down shift and click up to four column headings to sort by multiple factors.

You can export data from this tab to Microsoft Excel files. All exported files will be saved in the folder *C:\Optim.Client\Export*. The spectra displayed will have been calibrated by default using the four calibration procedures described. To export a calibrated spectrum to Excel before performing any analysis:

- Right click on the spectrum in the list and choose *Export Calibrated Spectrum*.

A subset of spectra can also be exported by well, temperature, measurement or all spectra can be exported to Excel. To export many spectra:

- Click the EXPORT button. Clicking an option in this window will send an exported file to the designated folder.
- Close the dialogue box when you are finished by clicking the cross in the top right hand corner.

You can also opt to export the raw data. Raw data is data collected directly from the CCD camera without wavelength, device response or background corrections having been applied. Raw data is always stored in the database so that the user can load an old set of data and export the raw file at any time after the acquisition.

To export raw data:

- Right click on the spectrum in the list and choose *Export Raw Spectrum*.
- Alternatively, click the EXPORT button. In the dialogue box, select a single spectrum to export or click *Export All Raw Spectra*.
- Close the dialogue box when you are finished by clicking the cross in the top right hand corner.

To download the *.xml* file:

- Click on the blue ID of any of the spectra listed to open an external dialogue box with the options to *open* or *save* the file.

These files will be in an open *.xml* format, which contains all raw data, calibrated data and a header listing all the settings of the instrument at the time of acquisition. Any files accessed via these links will be saved in *C:\Users\...\Downloads*.

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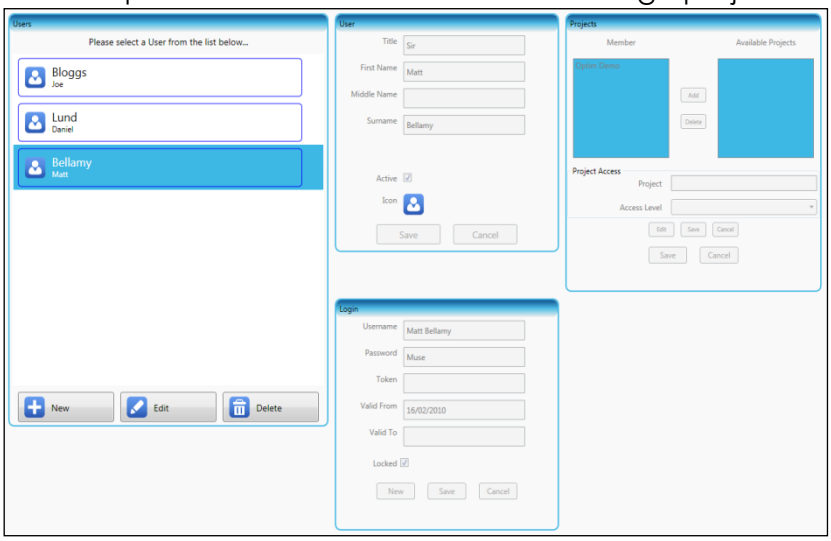
IX. USER MANAGEMENT

Users

This tab allows the Administrator logged into the "Optim Demo" to add users to the database, define the permission levels of the user and assign projects to the user.



New Users

To create a user:

- In the **Users** tab, you will see a list of the users in the left hand window. At the bottom of the window, click NEW.
- You will be prompted to enter a *username* for the new user.
- Enter the new user's personal details in the **User** window.
- In the **Login** box at the bottom you can edit the username and create a password for the new user (these are not case-sensitive).
- Click SAVE to save all the changes on this page.

Assigning Projects to Users

When assigning a Project to a user you must decide the user's level of access for each Project. It is advisable to have just one "Supervisor" for each Project and to assign all other users to the default "Analyst" access level. A Project Supervisor will be able to edit the details of the selected Project and grant access to other users but can also create, edit and delete their own projects. An Analyst level user can merely work on the Project and view its details.

To assign a Project to a User:

- In the **Projects** window of the **Users** tab, highlight a Project in the right-hand list and click ADD. The Project will appear in the *Allocated Projects* list on the left of this window.
- The default access level for a user is Analyst. Define the user's access level by selecting the Project and clicking EDIT in the access level box.

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Choose either *Analyst* or *Supervisor* from the drop down list. Click SAVE in this window.

- Click SAVE to save all the changes on this page.

Listed users can be edited, deleted or disabled. The Administrator will always be able to see a full list of the users and make changes to the list.

Deleting or Disabling User Profiles

On the occasion that you want to remove a user from the database, there are two options: to disable or delete the user.

- Disabling a User:

  To maintain access to the user's details but stop them from logging in, simply disable the login for that user by ticking the *Locked* box on their profile. Their name will still appear in the list of users but their login details will be disabled so that an error message appears if the user tries to log in.

- Deleting a User:

  Highlight the user's name in the list and click DELETE. This will deactivate the user so that their name is removed from the list of users and you will no longer be able to access their profile. Any data that the user collected will still be accessible by other users through the Project database.

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X. DATA MANAGEMENT

In the Optim Client software there are two types of data: *Global* and *Project-specific*. Most of the data that an Analyst-level user encounters is *Project-specific*. However, the Administrator can also change *Global* data.

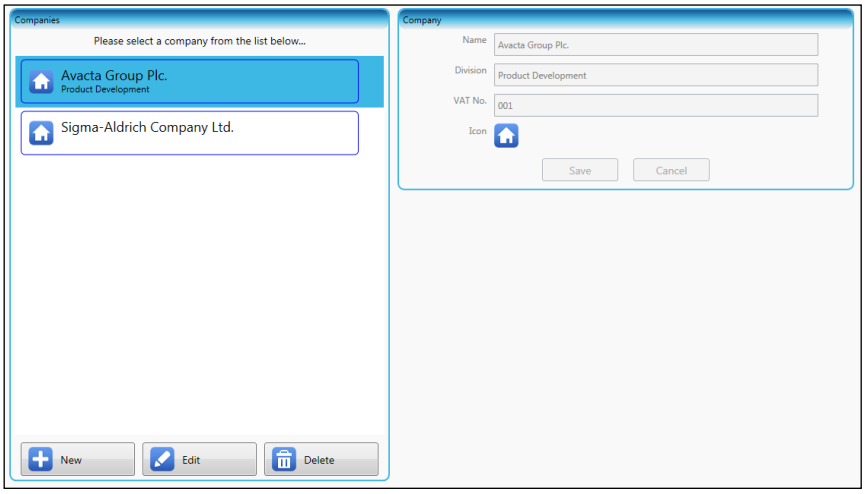
The list of users is an example of *Global* data. The database contains a list of users as created by the Administrator. When logged in, the Administrator can always edit and view the full list of users. Similarly, the **Companies**, **Projects**, and **Buffers and Additives** sections contain *Global* data.

Samples and Experiment **Runs** are examples of *Project-specific* data. These lists only contain data that is specific to the chosen Project.

The following sections describe how the Administrator might use each tab to manage their data.

Companies

This tab allows the Administrator to keep a record of the companies that are associated with an investigation, for example, companies that provide samples or experiment templates. As a default, Avacta Group PLC will be listed. An important function of this list is to identify the source of bought samples in the database.



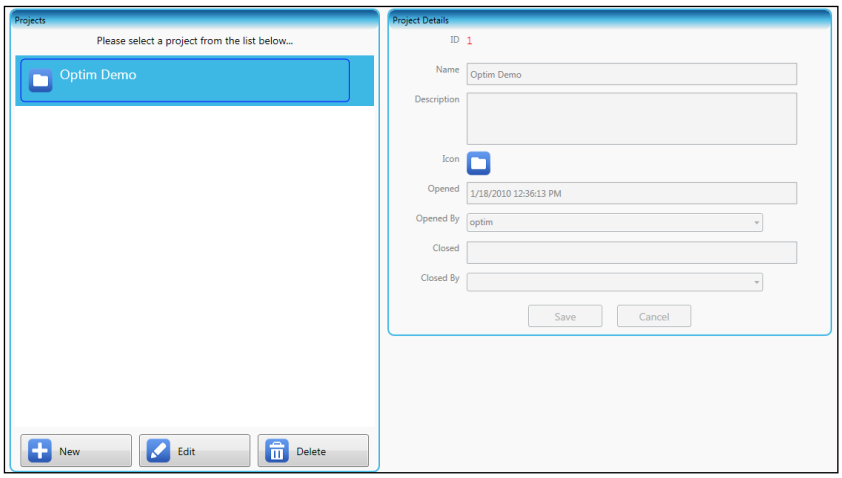
To add a company:

- Click NEW.
- Fill in the name and details of the company in the window on the right.
- Click SAVE.

This company will now be listed in a drop-down list on the **Samples** tab so that their records can be kept on the database.

Projects

The Administrator can use this tab to create, edit and delete projects in the database. Supervisors can also create new projects in this tab. The **Projects** tab contains a list of all projects that have been created. Click on a Project to load all the settings associated with it, or to edit the details.



To create a new Project:

- Click NEW.
- Enter a title and description for the new Project. The date that the Project was created should be filled in automatically. Click SAVE.
- Go to the **Users** tab and choose the users and access levels for your Project.
- Go to the **Samples** tab to create a record for each molecule you intend to study within this Project.

To view an existing Project:

- Highlight the Project in the list on the left. You will be able to see which user logged into this Project most recently and when the Project was created.
- Click EDIT to edit the details of the Project.
- To view old data or add new data to this Project, go to the **Experiments** tab and LOAD a Run or create a NEW Run respectively.

To gain a better understanding of the data management structure within the software, please refer to the flowchart.

Buffers and Additives

The Administrator controls the global list of buffers and additives that are available to the user. To add a new *Buffer* or *Additive* to the database, use the **Samples** tab.

- In the **Samples** tab, click NEW BUFFER.
- A window will pop up prompting you to type in the name and details of the new *Buffer*.

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- Click SAVE to add the new buffer to the database. This buffer will now appear in the buffer drop-down list on the **Formulations** tab.
- To input a new *Additive* to the database, click NEW ADDITIVE and follow the same procedure.

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XI. EXPERIMENT SETTINGS

This section contains the details of all of the preset functions in the software. Here you can find definitions of the different types of Experiment, discussion of the temperature step function, explanations of the calibrations that are applied to the data, and information about the analysis macros that are performed at primary and secondary level.

Experiments

The user has been provided with four templates for experiments (listed here). Within a Project, any or all of the experiments can be performed. Each of the templates has predefined settings that are suitable for a typical stability investigation. These are described below. (The capability to create user-defined experiments will be included in a future release of the software).

1) Stepped Thermal Unfolding Study

Aim: To determine the formulation that provides the greatest resistance to thermally-induced denaturation of proteins with increasing temperature.
Method: Probing the intrinsic fluorescence and scattered UV light while stepping up the temperature of the sample. The position and shape of the intrinsic fluorescence spectrum changes as the protein unfolds. The sample is heated using a stepped profile. **All the samples are equilibrated at the measurement temperature and then held at this temperature while they are measured. The temperature is then incremented, the samples are equilibrated, held, and measured again**.

2) Isothermal Unfolding and Aggregation Study

Aim: To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins at a constant elevated temperature over a period of time.
Method: Examining the intrinsic fluorescence and UV and blue scattering as a function of time whilst the temperature is held at a constant, elevated level. When under thermal stress, the proteins begin to unfold and aggregate. This is indicated by an increase in the intensity of the scattered light and changes in the fluorescence spectra. By using a UV laser for high sensitivity measurements and a higher power blue laser with a broader focus, aggregate size differentiation can be achieved.

3) Stepped Thermal Unfolding and Aggregation Study

Aim: To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins with increasing temperature.

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Method: Examining the intrinsic fluorescence and UV and blue scattered light while stepping up the temperature of the sample. When under thermal stress, the proteins begin to unfold and aggregate. This is indicated by an increase in the intensity of the scattered light and changes in the fluorescence spectra. By using a UV laser for high sensitivity measurements and a higher power blue laser with a broader focus, aggregate size differentiation can be achieved. The sample is heated using a stepped profile. **All the samples are equilibrated at the measurement temperature and then held at this temperature while they are measured. The temperature is then incremented, the samples are equilibrated, held, and measured again**.

4) Linear Thermal Unfolding and Aggregation Study

Aim: To determine the formulation that provides the greatest resistance to thermally-induced denaturation and aggregation of proteins with linearly increasing temperature.
Method: Examining the intrinsic fluorescence and UV and blue scattered light while linearly increasing the temperature of the sample. When under thermal stress, the proteins begin to unfold and aggregate. This is indicated by an increase in the intensity of the scattered light and changes in the fluorescence spectra. By using a UV laser for high sensitivity measurements and a higher power blue laser with a broader focus, aggregate size differentiation can be achieved.

Temperature Control

When choosing the temperature control settings, it may be useful to consider the following information about the logic of the software. The most common use of the temperature control is for a ***Stepped Ramp Type Experiment***. It is important to note first of all, that the temperature will not vary continuously, as implied by the word "ramp" but is in fact more like a staircase. A ***Linear Ramp Type Experiment*** does exactly the same thing but the temperature does vary continueously, at a programable ramp rate, while measurements are carried out continuously.

The user decides the ***Start*** and ***Stop*** temperatures and the ***Step Size***, and the instrument performs a complete set of measurements on every well at each set temperature point. Decreasing the step size results in a larger set of data which will of course take longer to collect. You might want to bear this in mind if you have chosen a small step size and have a large number of samples on the hot plate, as the samples will be held at elevated temperatures for as long as it takes to get all the measurements. This may inflict thermal stress and cause unexpected changes to the properties of the protein or macromolecule under investigation as the last samples scanned will have been held at temperature for longer than the first.

The temperature that is displayed in the ***Current Temp*** box represents the temperature inside the micro-cuvette. The actual temperature of the hot plate is measured directly and a calibration function is applied to find the temperature of

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the sample in the cuvette. This is accurate to within 0.5 degrees over the full temperature range.

When a temperature step is made, the Peltier device heats the plate until the sample reaches the new set temperature within the range of the factory-set tolerance, which is typically 0.2 °C. As soon as the temperature is within this range, a timer is triggered. The instrument waits for the designated ***Hold Time*** before beginning the first exposure. It is recommended that the user set a hold time of at least 30 seconds, as this allows the samples to reach the set temperature.

In a linear ramp the user can set a **plate hold time** and a **well hold time**. The well hold time is an option for you to add a delay between moving onto and measuring the next well. The plate hold time is a delay between measuring the last well and starting to measure the first well again. So the temperature will increase at a rate and the instrument will constantly move between the wells acquiring data as fast as it can. If you want to, you can make it pause after either each well or each complete measurement of the 3 MCAs or neither.

Calibrations

When data is captured using the program, four types of calibration are applied to the raw 'camera capture' data before the spectra are displayed in the **View Spectra** tab. It is possible to export to data from this tab to Excel without these calibrations by choosing *Export Raw Data* from the menu options.

The four calibrations are applied in the following order:

1) **X-axis Calibration**: Converting pixel numbers into an appropriate wavelength scale.
2) **Background Subtraction**: Subtracting a CCD camera-imposed background so that zero intensity means no photons are hitting the camera.
3) **Device Response Calibration**: Correcting for the variation in efficiency of the CCD's response to a range of wavelengths so that the intensity of the spectrum is proportional to the number of photons.
4) **Laser Power Calibration:** Calibrating the laser power over the full set of measurements to account for small variations in power over long time periods.

Of the four calibrations performed, two are automatic and two are pre-calibrated. Functions (1) and (3) are configured by Avacta on installation and checked at every subsequent service. The four calibration procedures are explained in greater detail below.

1) X-axis Calibration

The CCD detector is an array of pixels. When a photon is incident upon a pixel, it causes a voltage change across the pixel. The spectrograph uses a grating to spatially separate light of different wavelengths by diffraction.

This spatially separated 'image' of the light leaving the spectrograph is focused onto the pixels of the CCD with different pixel positions corresponding to different

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wavelengths. The spectrograph has a focal length, which in association with the size of the pixels in the CCD and the diffraction grating dispersion determines the pixel resolution. (Bear in mind that the achievable spectral resolution will also be affected by the choice of slit width. A wider slit increases the light throughput at the expense of the final resolution.)

Inside the spectrograph is a diffraction grating. Light incident on this grating at a known angle (angle of incidence) is diffracted and reflected at an angle (diffraction angle) which varies depending on the wavelength of light and the density of the grating. The centre wavelength is defined as the wavelength whose diffraction angle causes it to be imaged in the centre of the CCD array. Regardless of the numerical value of this centre wavelength, the sum of the angle of incidence and the diffraction angle is a constant. This is called the *inclusion* angle.

The CCD detector and spectrograph are joined together physically within the Optim 1000. To account for any misalignment in the connection, an offset angle (the *detector* angle) is determined.

As such, in order to know which wavelength is being represented by each pixel it is necessary to know:

- The wavelength centred on the CCD (λ_{centre})
- The inclusion angle between the photons incident on the grating and the diffracted photons which will reach the centre of the CCD (θ_i)
- The detector angle to compensate for mechanical tolerances (θ_d)
- The focal length (*f*) of the spectrograph and the pixel size (x) on the CCD
- The number of lines per millimetre on the grating (d)

These are related in the following manner* to obtain the wavelength, λ, at a numbered pixel, *p,* from the centre of the CCD.

$$\lambda = d\sin\left(\arcsin\left(\frac{\lambda_{centre}}{2d\cos(\theta_i/2)}\right)-\frac{\theta_i}{2}\right)+$$
$$d\sin\left(\arcsin\left(\frac{\lambda_{centre}}{2d\cos(\theta_i/2)}\right)+\frac{\theta_i}{2}+\arctan\left(\frac{px\cos\theta_d}{f+px\sin\theta_d}\right)\right)$$

Initially, only the pixel size and number of lines per millimetre on the grating are known. By using a calibrated light source with a known wavelength output (a spectral line source) it is possible to calibrate the spectrograph such that the wavelength which is centred on the detector is known.

Having measured this, it is possible to measure the pixel at which a known wavelength occurs, and iteratively fit this information to the formula above to obtain all the parameters needed to correctly scale the x-axis.

* Further information about the grating equation used to obtain this information and the optics of a spectrograph can be found in Hecht - "*Optics*" published by Addison Wesley, 2001.

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2) Background Subtraction

The CCD camera inside the Optim 1000 imposes a dark background signal of ~600 counts onto each acquired spectrum. This background level is subtracted from the raw data in the following manner.

- At the start of an experimental run, the Optim 1000 captures a dark image with no spectral information.
- The signal is measured at two points on the spectrum and a straight line is fitted between these points to obtain an average background measurement.
- This is then subtracted from every acquired spectrum.

3) Device Response Calibration

The Optim 1000 provides the user with a true representation of the fluorescence response of a protein sample. In order to do this, it is calibrated to ensure that this wavelength dependence on the digitised signal from the CCD camera does not affect the quality of spectra using a device response function.

The pixels within the CCD in the detector contain electrical elements which generate an electric current when illuminated with light. The efficiency of this process is wavelength dependent, so if a broad spectrum of light, with each wavelength having the same intensity, was incident on the CCD the resulting digitised spectrum would not be a flat line, but would display a variation in the number of counts measured.

A device response calibration function is obtained by using a calibrated light source. It is shone into the instrument and the raw data acquired is compared to the known spectrum of the light source. The resulting function is stored on the Optim 1000 database and is automatically applied to all acquired spectra.

4) Laser Power calibration

The UV laser used within the Optim 1000 is a stable light source. However, towards the end of a laser's life the power output can begin to fluctuate. As such, a final calibration step is present in the software. The laser power at the start of an experimental run is recorded, and each subsequently acquired spectrum is normalised such that the laser power at the time of the measurement is the same as that at the start of the Run.

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THE OPTIM ANALYSER HANDBOOK

The Optim Analyser v1.5 is a software package designed and built to automatically and robustly analyse data generated on an Optim 1000 instrument using the client software. It can be installed on any Windows based personal computer, and for details of doing so please refer to the relevant section of this manual.

XII. GETTING STARTED

The Optim Analysis v1.5 software features two modes of operation

- **Wizard mode** – this mode allows fast and automated analysis of the data using a simple wizard based system. (This is the default view.)
- **Advanced user mode** – allows the user to analyse and display a large selection of data in a huge variety of different ways using a plethora of functions and user definable methods.

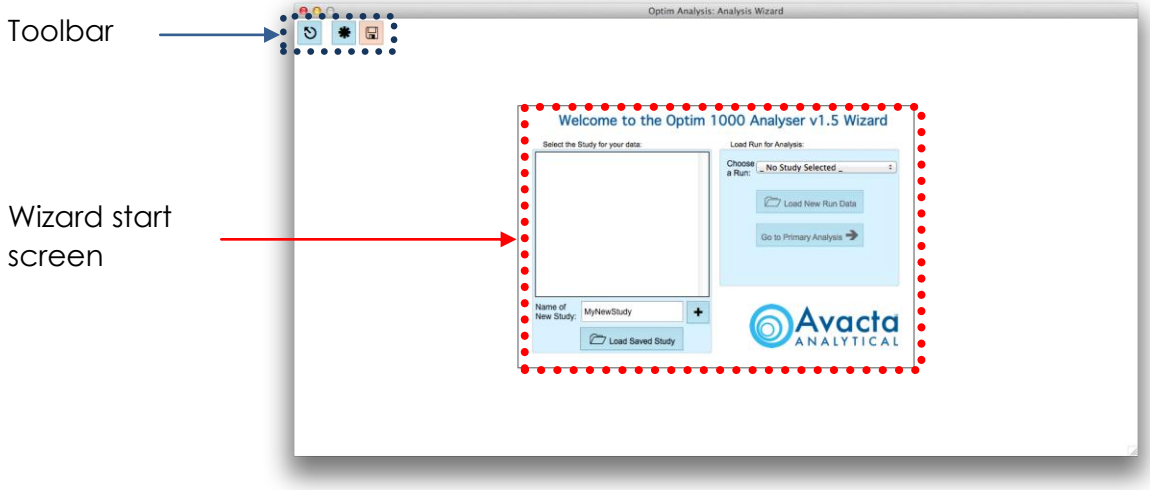
Data within the system is stored within "Studies". Each study can contain many runs that can be analysed. The runs can be loaded from any Optim 1000 instrument that is running version 1.5 of the Optim Client software.

A study in the Optim Analysis software is similar to a project in the Optim Client software. The two main differences other than the name, is that a study can be passed around between users and computers and viewed/edited at will.

A first look...

To start the Optim Analysis software ensure that the USB dongle is inserted and double click on the Optim Analysis icon that is on the desktop.

This will start the software and you will be presented with a splash screen and then the default application screen will be displayed. The default view of the software is the wizard mode start screen.



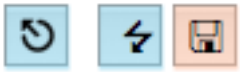
The wizard mode toolbar provides some quick shortcuts to tasks that you may perform on a regular basis.

They have the following function

Symbol	Action
	Shutdown Optim Analyser software
	Go to the Advanced analysis mode
	Save the currently loaded analysis study

Advanced mode

In advanced mode, the screen is divided into tabs and sub-tabs for performing different tasks. There is also a toolbar to allow you to perform common tasks easily.



The symbols in this toolbar have the same meaning as they do in the wizard mode, with an additional button, ↯, which returns to the wizard.

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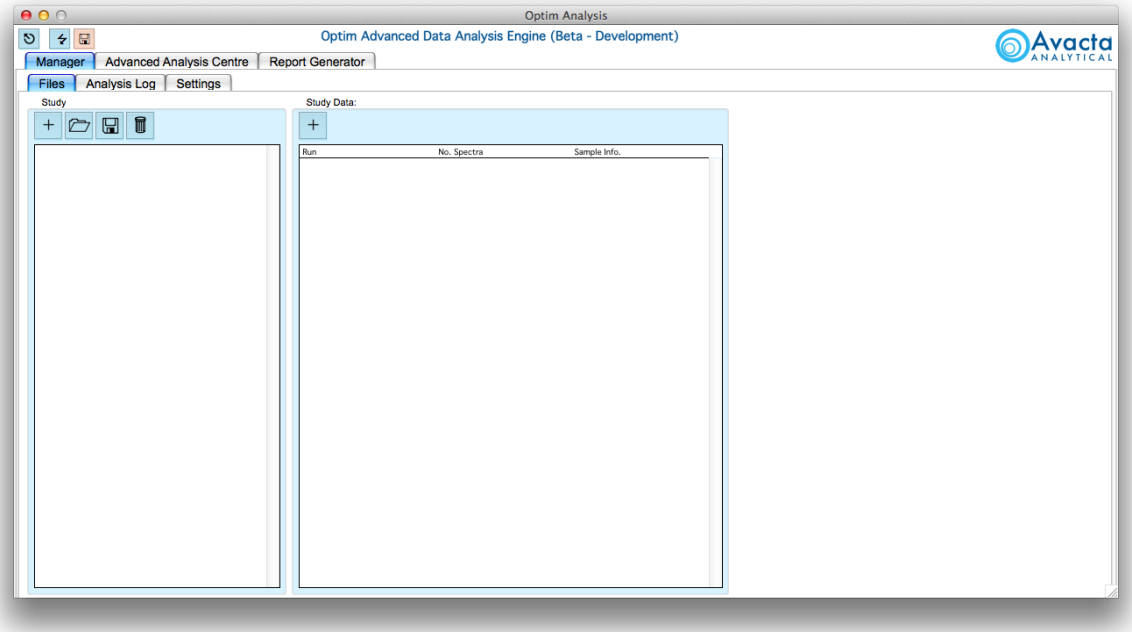
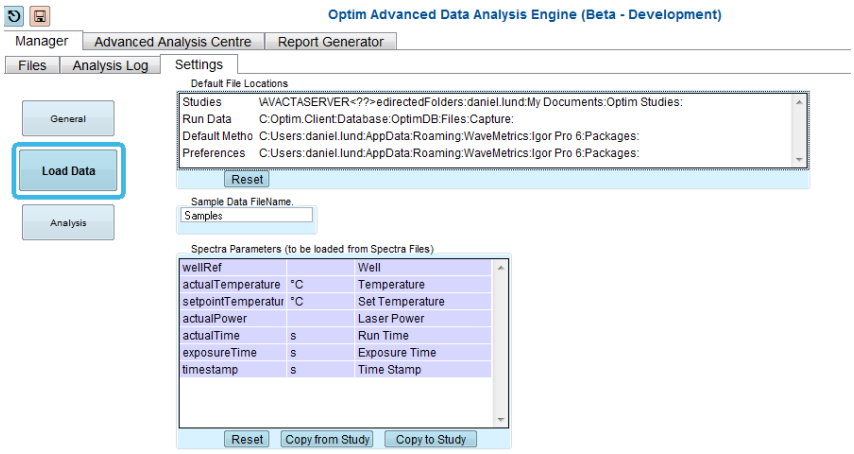


In this mode, data structures can be created and removed, spectra can be loaded and settings changed through the Manager tab. Analysis can be performed, and custom mathematical methods created through the Advanced Analysis Centre, and if one wishes to eschew obfuscation the Report Generator can be used to generate reports.

Before you can start to analyse data you need to configure your settings. In the Manager tab, select the Settings sub-tab to display the current settings.



To change where data is loaded from:

- Select the **Load Data** option on the left hand side to change the preferences
- Under "Default File Locations" change the Studies and Run data locations to reflect their paths.

The differences between the three data are:

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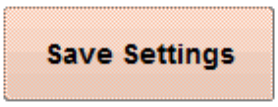
- Studies – collections of runs and analyses that are saved by the Optim analysis software.
- Run data – the raw spectra generated by the Optim Client.
    - If this software is installed on the desktop PC then this path should be C:Optim.Client:Database:OptimDB:Files:Captures:
    - If you are running the software on a separate computer then this path should point to the location to which you are copying data.

You can save these options by clicking SAVE SETTINGS in the **General** tab.



Choosing the analysis mode you want to use

With two (wizard or advanced) analysis modes built into the system, making the correct choice for your needs is important to get right. Luckily the analysis engine underneath is the same regardless of your choice, so you can switch between them interchangeably at any time.

However, when choosing how you wish to use the software it is useful to refer to the information below to ensure that you are appropriately informed and able to choose wisely.

Use the Analysis Wizard if you wish to:

- Analyse data from a variety of samples within a single run using the same analysis method.
- Obtain unfolding and aggregation curves versus either temperature or time depending on the experiment type you are doing.
- Calculate melting temperatures from unfolding data obtained from fluorescence.
- Calculate aggregation onset temperatures from static light scattering.
- Calculate trends relating to well-to-well variations.

Use the Advanced Analysis Package if you wish to:

- Analyse data from samples which have different spectral characteristics (i.e., tyrosine and tryptophan) using different analysis methods.
- Change the default analysis methods to better suit your requirements.
- Develop new analysis methods capable of performing a wide range of analytical tasks.
- Check the results of secondary analysis by looking at the data superimposed with the primary data.
- Analyse data from multiple runs simultaneously.

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XIII. THE OPTIM ANALYSIS PHILOSOPHY

In this section the data flow around the system and the definition of the different analysis levels are defined. There are three levels of analysis in the software. Each of the levels has three attributes, inputs, methods and outputs.

	Primary	Secondary	Tertiary
Scope	Extract parameters from measured spectrum that relates to either mean molecular mass of solution contents or the tertiary structure of the protein in the sample	Extract melting points, onset temperatures or rate constants from unfolding and SLS curves generated by the primary analysis	To look at variations from sample to sample, for the purpose of looking for trends under different physical conditions, be it pH, concentrations, etc
Input	Spectra consisting of intensity vs wavelength	Unfolding curves and aggregation curves, composed by displaying the primary analysis results against either temperature or time	T_m T_{agg} k_{obs} Primary analysis result at a particular temperature/time
Default methods	Integrated scattering intensity Lorenzian peak fit Barycentric mean Intensity ratio at 350:330	T_{agg} through edge detection T_m through differential against temperature k_{obs} through either single or double exponential fit	Linear regression Quadratic fit D_m through differential against concentration of excipient ΔG_{UN} and M-value fit
Default outputs	A value for each spectrum for the parameters extracted from the methods listed	T_m T_{agg} k_{obs}	D_m, ΔG_{UN}, M_{UN} Second virial coefficients Gradients and intercept

The data can be viewed in a data structure that is made up of:

1. A study
2. A run
3. A series of collections comprising either
    a. Spectra

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b. Primary analysis results
c. Secondary analysis results
d. Tertiary analysis results

Data that is stored within a collection is passed through a method and that generates output that can be added to a separate collection and analysed by a subsequent level of analysis.

Only the study is a rigid entity. A study can contain data from many runs (in the advanced analysis mode these can be analysed simultaneously). A collection can contain many different members (whether they are spectra, unfolding curves etc) and the members themselves can be in many different collections.

In the wizard mode, the concept of collections is of little importance, as the user is not able to define their content. All data is passed to the analysis engine in this case.

For further information about the default methods, the outputs and what they mean refer to later sections in this handbook.

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XIV. THE OPTIM ANALYSIS WIZARD

The Optim Analysis wizard is the default method for analysing data in the Optim Analyser. It allows a simple, few-click analysis experience using a selection of intelligent default analysis methods to display the results you want.

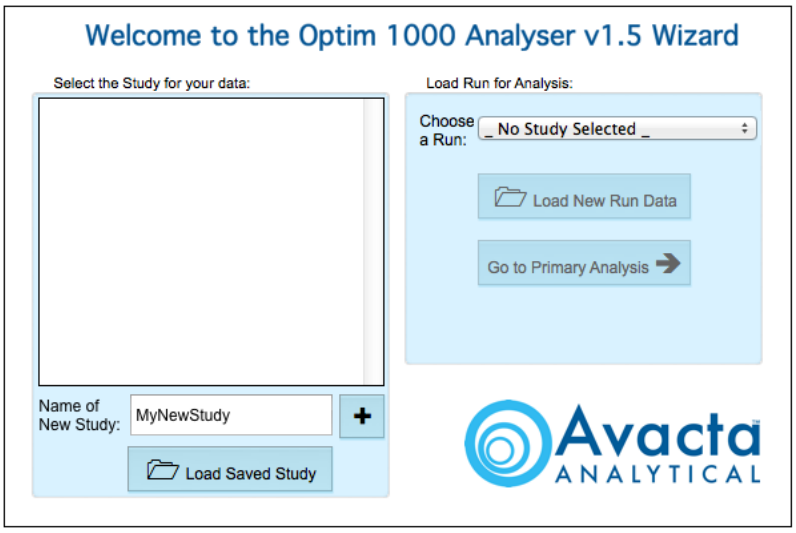
In this section of the handbook you will be guided through the process of loading some data, performing each of the analyses in turn and preparing a sample report.

Loading data into the wizard

The first step of any data analysis is to load data into the wizard to analyse. There are three available options:

1. Load newly acquired spectra into a new study.
2. Load newly acquired spectra into an existing study.
3. Load an existing study and reanalyse or view the previously analysed data.

The opening screen of the wizard presents the start of the process.


Welcome to the Optim 1000 Analyser v1.5 Wizard
Select the Study for your data:
Load Run for Analysis:
Choose a Run:
_ No Study Selected _
Load New Run Data
Go to Primary Analysis
Name of New Study:
MyNewStudy
Load Saved Study
Avacta ANALYTICAL

To load data into the system, either

1. If you need to create a new study to load your data first enter then name of the study and click on the "+".
2. If you are adding spectra from a run into an existing study then select the study and click LOAD NEW RUN DATA. This will open a pop-up box where you can select the run you want and click LOAD

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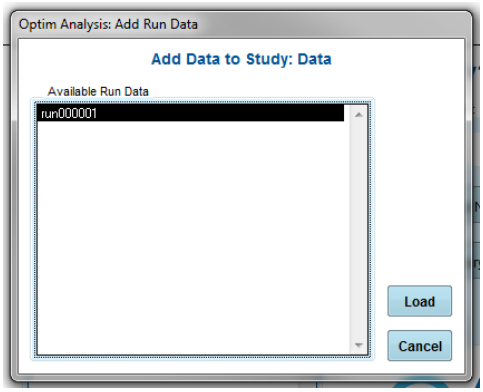
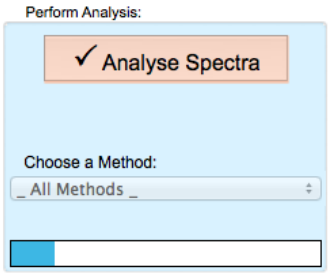


3. If the study is already loaded, select the study where your data is stored and choose a run from the dropdown box on the right.

When you have selected the run you wish to analyse then click on GOTO PRIMARY ANALYSIS➔ to go to the next stage of the analysis.

Performing Primary Analysis

The primary analysis can be completed by clicking ✓ANALYSE SPECTRA. A progress bar will appear underneath and indicate the progress of the analysis. By default the system will perform analysis using the default methods. If you wish to use a different analysis method – such as one defined and saved by an advanced user – you can select this in the dropdown menu.



When the formulation information was defined at the start of the experiment in the Client software the "Analyse" and "Reference" options were set to either TRUE or FALSE for each sample well.

Any sample well that had the Analyse option set to FALSE will not be analysed.

Any sample well that had the Reference option set to TRUE will be analysed and displayed, so long as the Analyse option was also TRUE. If any sample wells are present with Reference set to TRUE, then the system will recognise this as buffer and will present an option to automatically subtract buffer spectra from sample spectra before calculating the primary analysis. When this option is available you will see an option to enable buffer subtraction. When this is checked and set to ON there will be an additional two methods available in the dropdown box.

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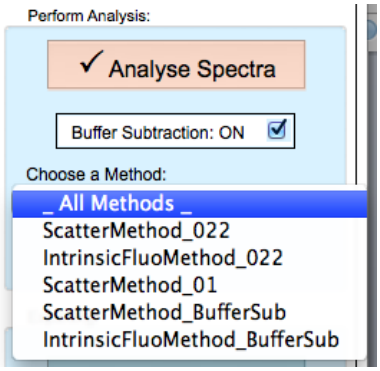


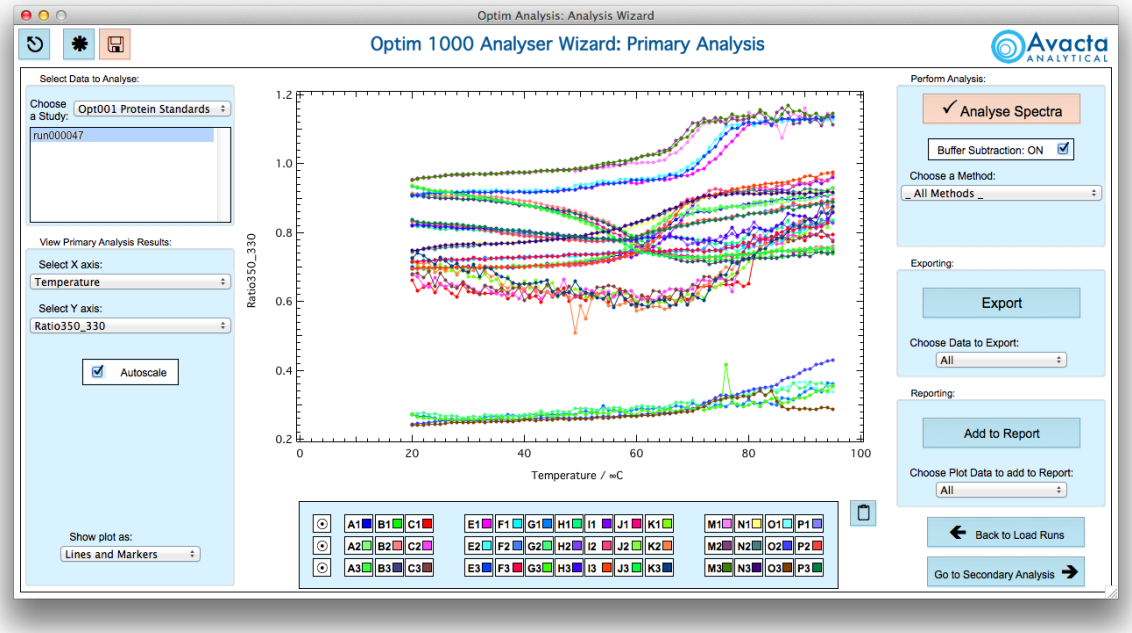
IMPORTANT For the software to be able to subtract buffer spectra from sample spectra it is essential that when using the wizard all the relevant spectra are present within the same run.

When the analysis is completed you can choose which dataset you wish to display by selecting an X axis and a Y axis.

You can change the scaling of the graph by unchecking the "Autoscale" box and manually editing the minimum and maximum X and Y axis values.

The graph can be displayed in one of three modes:

1. *Markers* – the graph will be displayed with points on the graph.
2. *Lines* – the graph will be displayed with no points but with lines
3. *Lines and markers* – the graph will be displayed with both markers and connected by lines.



The colour of the points and lines that are displayed on the graph corresponds to the key at the bottom of the graph. If you wish to add or remove results from a

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particular well from the graph you can click the coloured square next to the well reference in the key to do so. Clicking on one of the target buttons, ⊙, will toggle between hiding and displaying all wells in the selected MCA.

In the example shown, wells D1, D2, D3, L1, L2 and L3 had ANALYSE set to FALSE in the formulation information that was set within the client software and are thus not present in the analysed data.

To export the primary analysis results click EXPORT. If you select "All Wells" then the software will export all the data from all the wells of the primary analysis that is currently being viewed. If you select "Displayed" then it will export only the displayed graph data. Selecting "All Data" will export all wells for all primary analysis results.

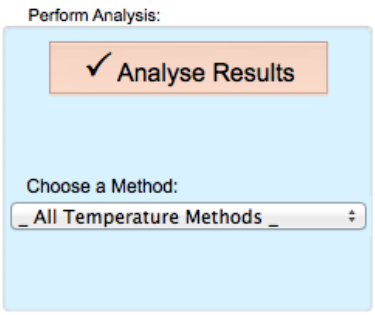
To add the primary analysis results to the report click ADD TO REPORT. Again, by selecting "All" then all the data will be added to the report in the Primary Analysis section. By selecting "Displayed" then only the displayed graph will be added to the report.

When you have finished the primary analysis click GOTO SECONDARY ANALYSIS ➔ to proceed onto the next level of analysis.

If you wish to change the run that you are analysing within the same study, you can do so by making the selection on the primary analysis screen. You can also switch between studies by choosing a study from the dropdown menu. To add new run data to the software, or add or load another study you will need to click ← BACK TO LOAD RUNS to do this.

Performing Secondary Analysis

After the completion of primary analysis, clicking on ANALYSE RESULTS will carry out secondary analysis. By default the software will use the default secondary analysis methods and auto-detect the type of experiment carried out. If you wish to use a different analysis method – such as one defined and saved by an advanced user – you can select this in the dropdown menu.



When the analysis is completed you can choose which parameter (for example transition temperature or onset temperature) from a particular dataset that you wish to display by selecting them from the dropdown boxes. You can display the data either by well reference (such as A1, B1 etc), by the sample name (such as Poly IgG or Lysozyme) or the sample details (which is the full information).

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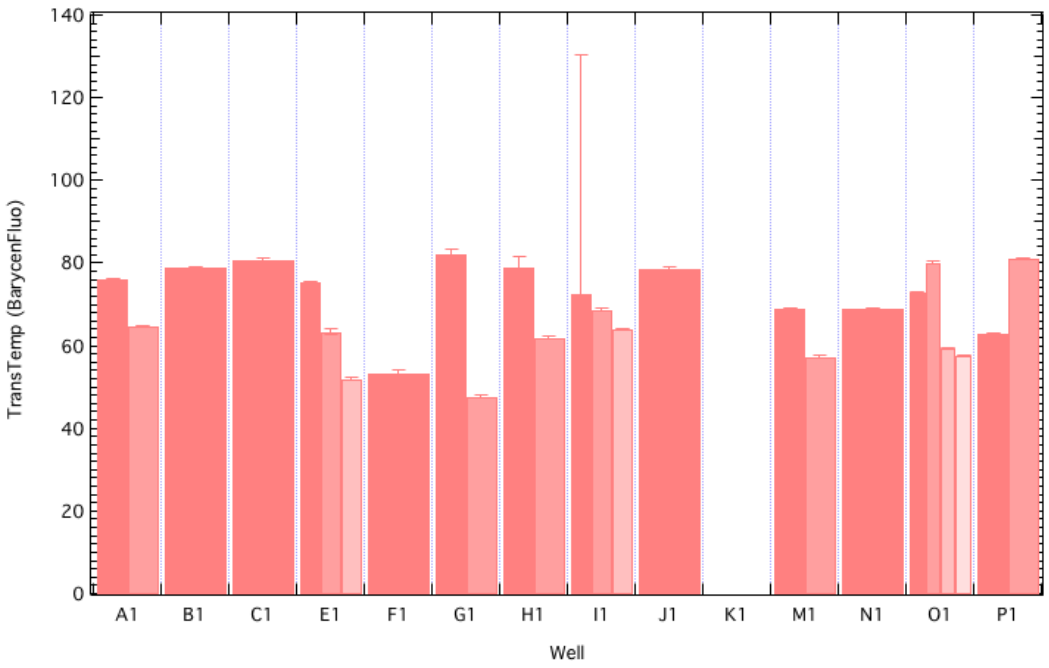
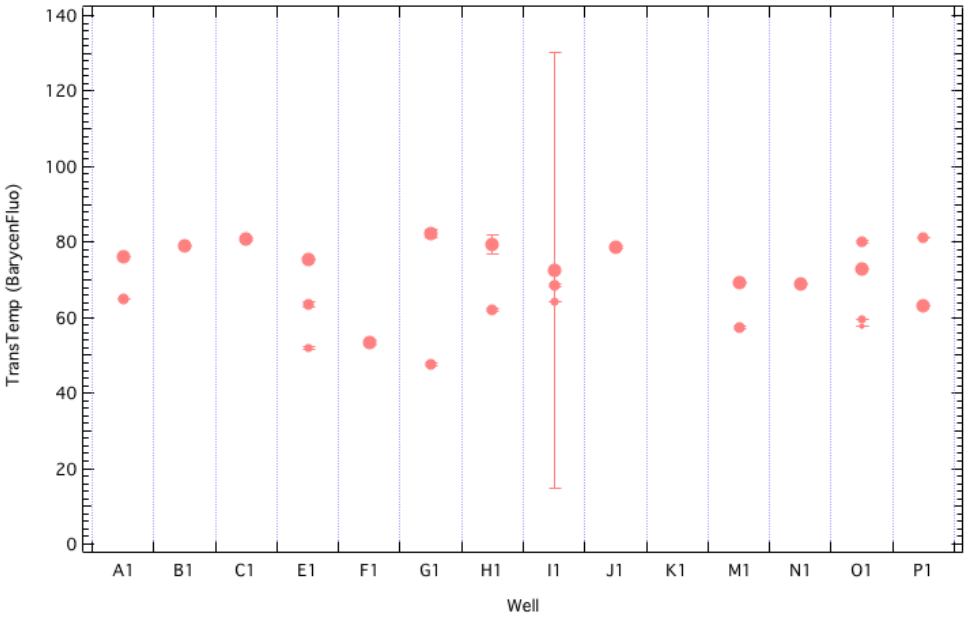
You can change the scaling of the graph by unchecking the "Autoscale" box and manually editing the minimum and maximum Y axis values.

The graph can be displayed in one of three modes:

1. Bar Chart – the graph will be displayed as a bar chart with the parameter indicated by the magnitude of the bar. In the case of multiple values each well will have multiple bars, as shown below. The most significant result will be shown in the graph first.



2. Markers – the graph will be displayed with points indicating the value of the parameter. Where there are multiple results they will all be overlaid. The most significant result will have a larger marker.



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3. Table – the data will be presented as a table showing the well reference, sample description and the values of the parameter.

The colour of the bars and points that are displayed on the graph correspond to the key at the bottom of the graph. If you wish to add or remove results from a particular well from the graph you can click the coloured square next to the well reference in the key to do so. Clicking on one of the target buttons, ⊙, will toggle between hiding and displaying all wells in the selected MCA. To export the secondary analysis result click EXPORT. If you select "All Data" then the software will export all the primary analysis results. If you select "Displayed" then it will export only the displayed graph data.

To add the secondary analysis results to the report click ADD TO REPORT. Again, by selecting "All" then all the data will be added to the report in the Secondary Analysis section. By selecting "Displayed" then only the displayed graph will be added to the report.

When the secondary analysis has been finished clicking GO TO TERTIARY ANALYSIS ➔ moves onto the next step of analysis.

Performing tertiary analysis

After completing the secondary analysis clicking ANALYSE RESULTS will perform tertiary analysis. This involves looking at the information provided in the formulation file during the configuration of the run in the client software, detecting whether there are any clear trends and analysing these.

If this information hasn't been entered correctly, or needs to be reviewed this can be accessed using the sample information editor by choosing *View and Edit Sample data* from the **Optim Utilities** menu at the top of the screen. (This will be covered in greater detail in the advanced analysis section of this manual.)

Typical trends that will be analysed are changes in any parameter with pH, concentration of sample or additive, analysis of replicates (to generate averages and standard deviations) and Debye plots with sample concentration to determine second virial coefficients.

The screen will give a graph with results displayed, and a key underneath that indicates what the curves and points are and give the result of any analysis.

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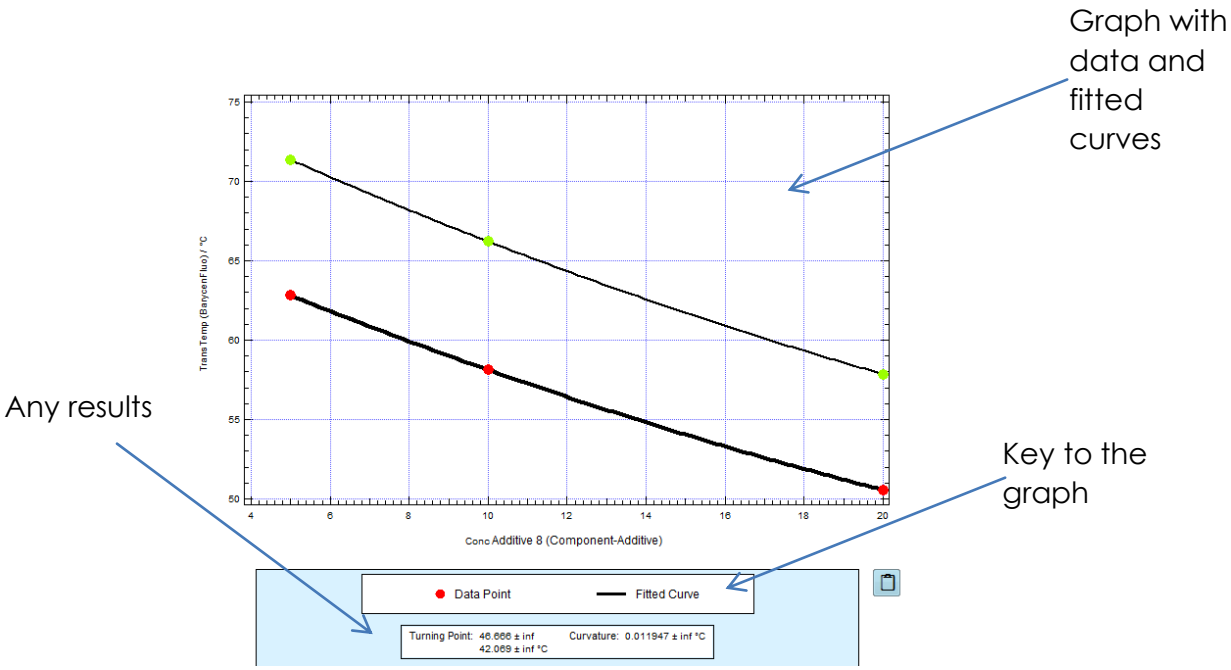
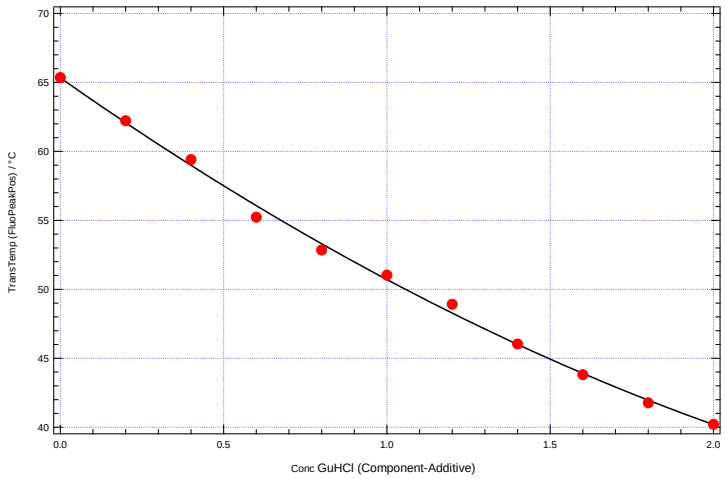

Graph with data and fitted curves
Any results
Key to the graph
TransTemp (BarycentFluo) / °C
Conc Additive 8 (Component-Additive)
Data Point
Fitted Curve
Turning Point: 46.666 ± inf 42.069 ± inf °C
Curvature: 0.011947 ± inf °C

The default analyses carried out here are:

1. Any trend analysis, such as choosing any Transition temperatures/aggregation temperatures vs concentration of protein/additive will be fitted with a quadratic function.



2. A primary analysis result, such as peak position at a particular temperature, plotted against concentration of a denaturant (such as GuHCl) will be fitted with a two-state equilibrium unfolding – yielding a free energy of unfolding (ΔG_{UN}), an M-value (which is the molar free energy change), and by dividing the free energy by the M value, a midpoint of unfolding.

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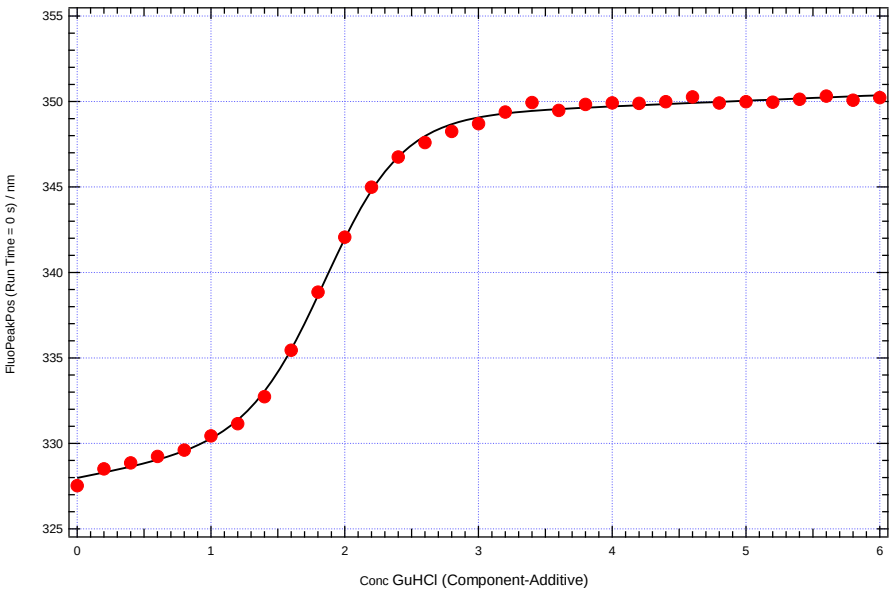
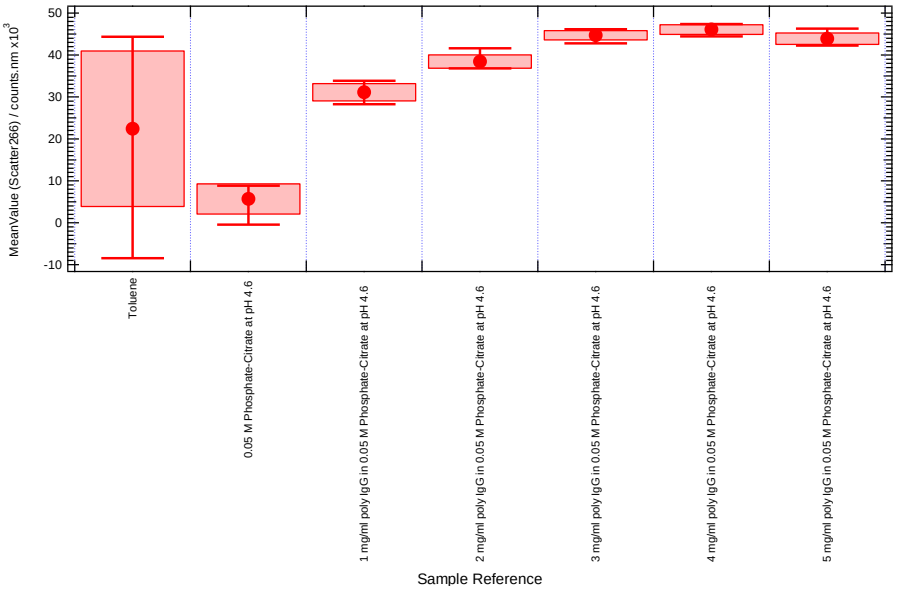


3. Plotting any datatype parameter against either sample reference or replicate number (as defined in the formulation information that is inserted in the client software) will display data representing the reproducibility of the measurement. In this case, the dot represents the average value, the box represents the statistical uncertainty, and the bar represents the range of the data.


MeanValue (Scatter266) / counts.nm x10^3
50
40
30
20
10
0
-10
Toluene
0.05 M Phosphate-Citrate at pH 4.6
1 mg/ml poly IgG in 0.05 M Phosphate-Citrate at pH 4.6
2 mg/ml poly IgG in 0.05 M Phosphate-Citrate at pH 4.6
3 mg/ml poly IgG in 0.05 M Phosphate-Citrate at pH 4.6
4 mg/ml poly IgG in 0.05 M Phosphate-Citrate at pH 4.6
5 mg/ml poly IgG in 0.05 M Phosphate-Citrate at pH 4.6
Sample Reference

4. Plotting a datatype of Kc/R0 (only available in certain circumstances) against sample concentration will display a Debye plot. Default parameters for solvent refractive indices will be used in this analysis (see section in advanced analysis for more information about performing these types of measurements).

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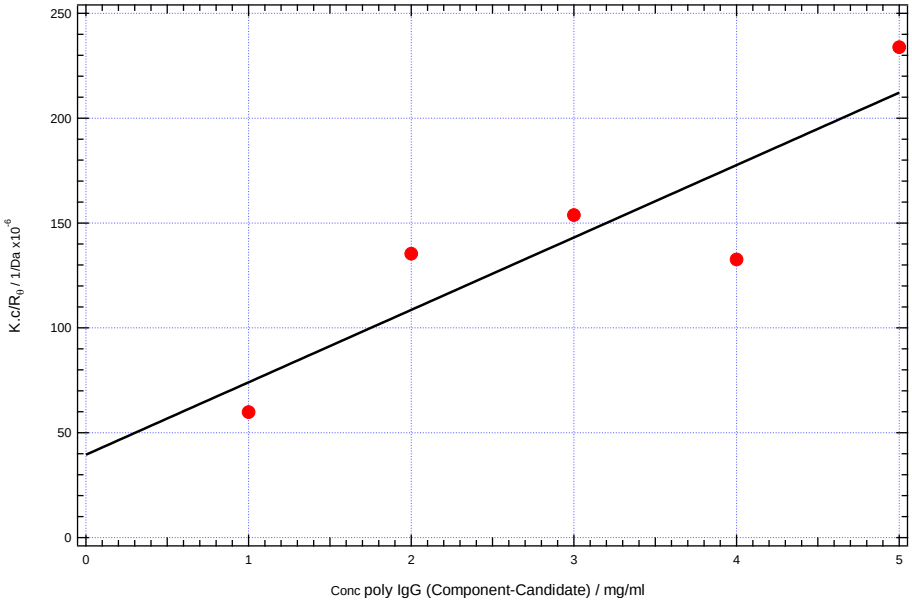
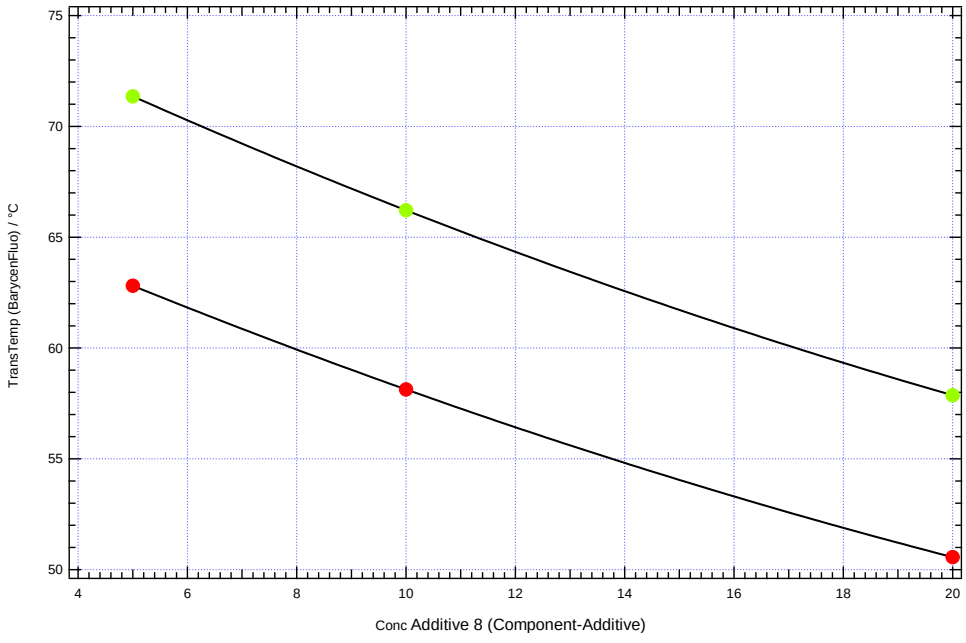


There are a large number of potential graphs that can be displayed here. Choosing to display the results as a table will display all results for a particular data type and x-axis combination.

In some cases, in particular with Debye plots or trend analyses, there may be multiple trends occurring in the same dataset. For example, if a formulation study is being done with concentration gradients of NaCl in both citrate phosphate and phosphate buffered saline. There will therefore be two sets of data, each of which shows variations in transition temperatures (etc) with concentration of NaCl.

As such, choosing a subset from the subset menu will enable the user to choose which particular trend to display.

When there are two results for an individual trend (such as two transition temperatures) these will be displayed in different colours, as shown below.



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Hovering over each dataset shown will display the results for that particular transition temperature trend. When displayed as a table the different trends will be labelled as "Result No.".

Trend Parameter	SubSet	Result No.	Fit Type	Fit Parameter 1	Fit Parameter 2	Fit Parameter 3
Additive 1 (Component-Additive)	_ no data _	1	Quadratic	26.615 ± inf	51.866 ± inf °C	0.021947 ± inf °C
Additive 1 (Component-Additive)	_ no data _	2	Quadratic	21.045 ± inf	60.285 ± inf °C	0.035592 ± inf °C
Additive 2 (Component-Additive)	_ no data _	1	Quadratic	39.902 ± inf	44.661 ± inf °C	0.015613 ± inf °C
Additive 2 (Component-Additive)	_ no data _	2	Quadratic	53.008 ± inf	49.545 ± inf °C	0.010233 ± inf °C
Additive 3 (Component-Additive)	_ no data _	1	Quadratic	694.04 ± inf	-61.556 ± inf °C	0.0002667 ± inf °C
Additive 3 (Component-Additive)	_ no data _	2	Quadratic	-163.79 ± inf	121.55 ± inf °C	-0.0016975 ± inf °C
Additive 4 (Component-Additive)	_ no data _	1	Quadratic	25.232 ± inf	53.322 ± inf °C	0.020793 ± inf °C
Additive 4 (Component-Additive)	_ no data _	2	Quadratic	20.839 ± inf	60.617 ± inf °C	0.035587 ± inf °C
Additive 5 (Component-Additive)	_ no data _	1	Quadratic	-3.7958 ± inf	65.462 ± inf °C	-0.014173 ± inf °C
Additive 5 (Component-Additive)	_ no data _	2	Quadratic	-29.311 ± inf	80.649 ± inf °C	-0.0065334 ± inf °C
Additive 6 (Component-Additive)	_ no data _	1	Quadratic	-43.998 ± inf	83.093 ± inf °C	-0.0082974 ± inf °C
Additive 6 (Component-Additive)	_ no data _	2	Quadratic	-35.7 ± inf	89.534 ± inf °C	-0.010338 ± inf °C
Additive 7 (Component-Additive)	_ no data _	1	Quadratic	18.768 ± inf	65.783 ± inf °C	0.0033437 ± inf °C
Additive 7 (Component-Additive)	_ no data _	2	Quadratic	12.167 ± inf	74.834 ± inf °C	0.010007 ± inf °C
Additive 8 (Component-Additive)	_ no data _	1	Quadratic	46.666 ± inf	42.069 ± inf °C	0.011947 ± inf °C
Additive 8 (Component-Additive)	_ no data _	2	Quadratic	47.386 ± inf	48.204 ± inf °C	0.012887 ± inf °C
Additive 9 (Component-Additive)	_ no data _	1	Quadratic	45.031 ± inf	47.598 ± inf °C	0.00952 ± inf °C
Additive 9 (Component-Additive)	_ no data _	2	Quadratic	140.62 ± inf	11.199 ± inf °C	0.0032401 ± inf °C
Additive 10 (Component-Additive	_ no data _	1	Quadratic	22.273 ± inf	62.416 ± inf °C	0.010113 ± inf °C
Additive 10 (Component-Additive	_ no data _	2	Quadratic	26.181 ± inf	69.172 ± inf °C	0.0098337 ± inf °C

Choosing to display the data as a table puts the results of all tertiary analysis for all subsets into a single table.

As before, all results can be copied to clipboard, exported to file, or inserted into the report.

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XV. ADVANCED DATA ANALYSIS MODE

The advanced analysis mode of the Optim Analysis software is capable of analysing the data generated on the Optim Client in a large variety of ways, both through default analytical methods that have been developed by Avacta Analytical and by user generated custom methods.

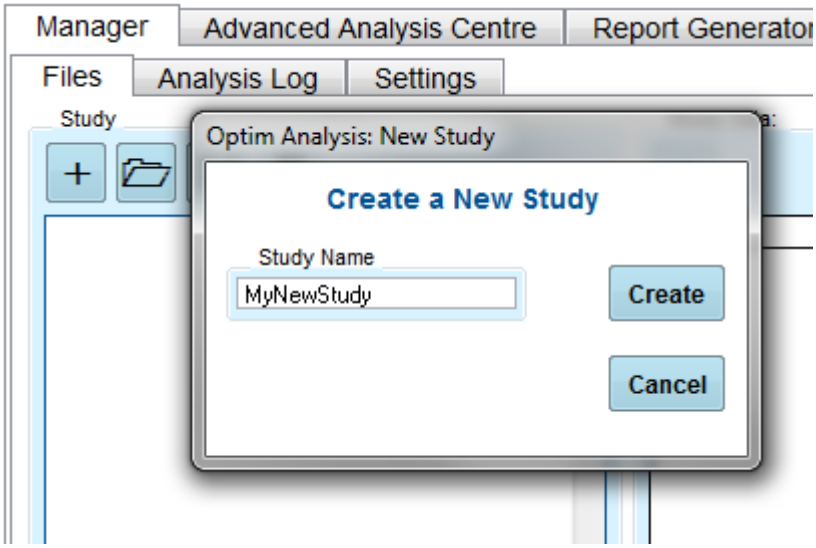
Data analysed in the wizard mode is compatible with the advanced mode and the two can be switched between at will.

Managing the software and Loading data

Loading data

The first step of performing data analysis is to load the acquired data. Create a new study to keep the data grouped (these could have the same name and contents as the projects in the Optim Client software).

To do this, select the **Manager** tab, and in the **Files** sub-tab click **+** in the Study section and give the new study a name. Then click CREATE.



To add spectra to our study, click + in the Study Data section. The software will list all the runs that are available to load. Select those that you wish to load (to select multiple runs hold down shift) and click LOAD. The system will then load, sort and automatically classify each of the spectra according to the information present in the formulation file.

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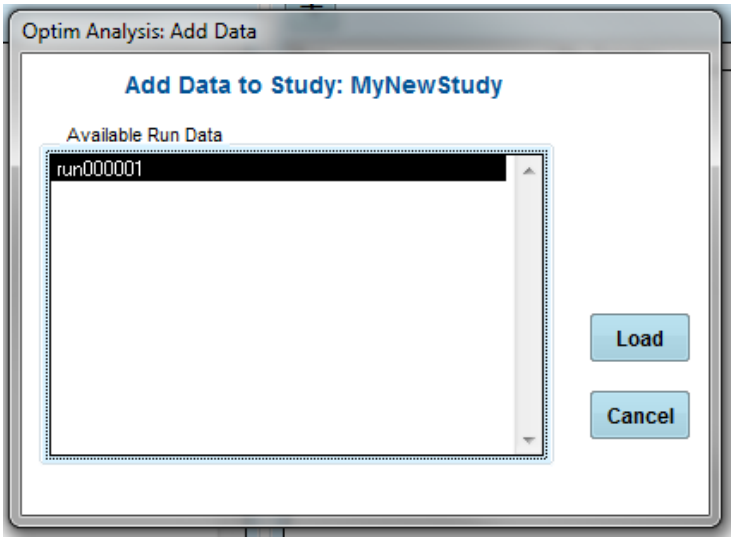
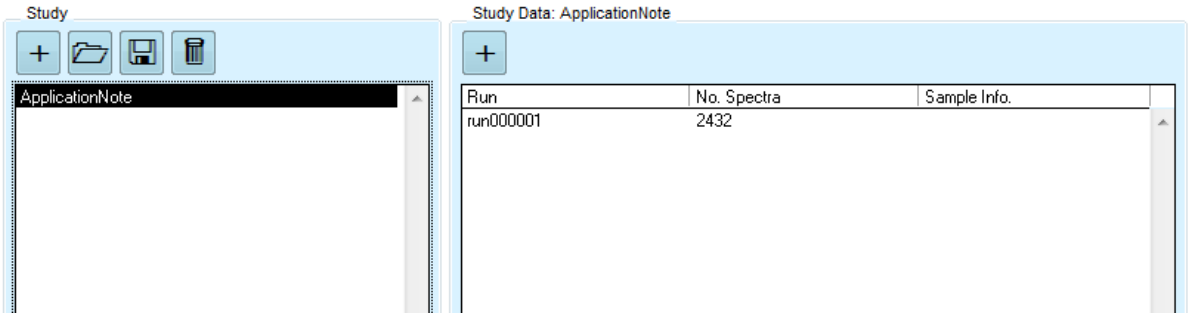


Once loading of the data is completed, the **Files** sub-tab will display the data that is currently loaded into the study.



To add further data, simply click + in the Study Data section.

The table below describes the functionality of the local buttons in the Study section of the files tab.

Symbol	Action
	Create a new study
	Open a saved study
	Save the currently open study
	Remove the current study from the software (note: this does not remove the study from the file system, it can still be loaded).

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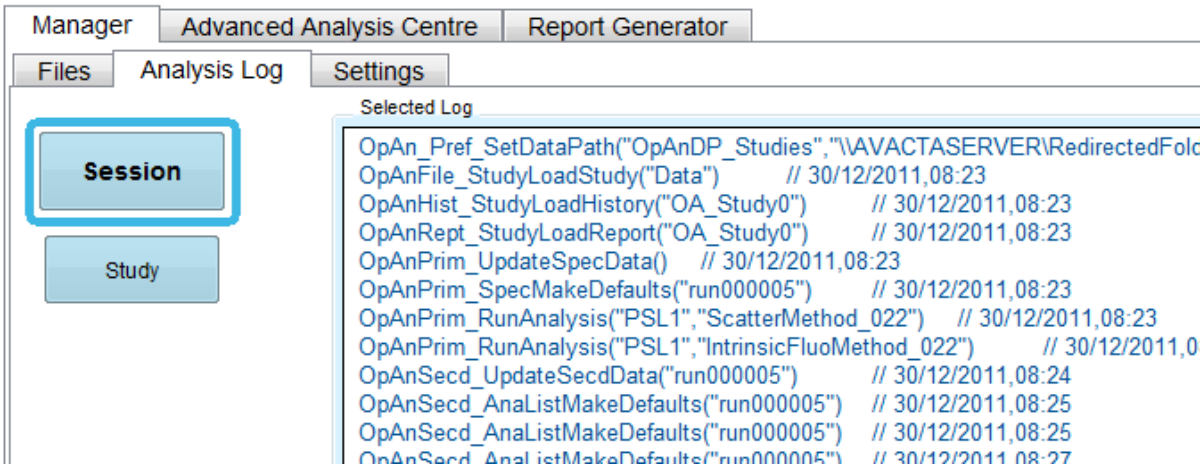
IMPORTANT - SAVE THE STUDY AT THIS STAGE OR YOU WILL HAVE TO RELOAD AND REANALYSE THE DATA.

TIP – You can also save the study by clicking the orange Save button on the toolbar

The Analysis log

Every action performed in the Optim Analysis software, whether in the wizard mode or in the advanced mode, is logged.

The **Analysis Log** sub-tab enables you to view the log of actions that have been performed in the study and in the current session.



Settings

A variety of settings can be configured in the **Settings** sub-tab. There are three classes of settings: GENERAL settings for miscellaneous settings; LOAD DATA settings for setting the file paths for loading data and selecting which parameters to extract from the spectra; ANALYSE settings to configure the analysis engine.

In GENERAL settings it is possible to edit the colours that are used to generate plots and graphs. The default colour scheme is a colour table featuring 48 different colours, each comprised of a value representing the contributions to the colour from RED, GREEN and BLUE. Each value can vary from 0 to 65535. Changing one of the values will change the colour respectively.

When you have changed any settings, in order to make sure that you don't have to change them again once you restart the software be sure to click the SAVE SETTINGS button in the GENERAL settings.

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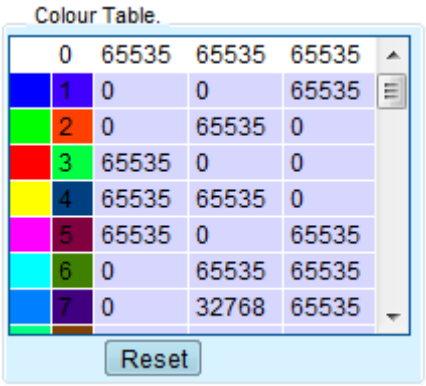
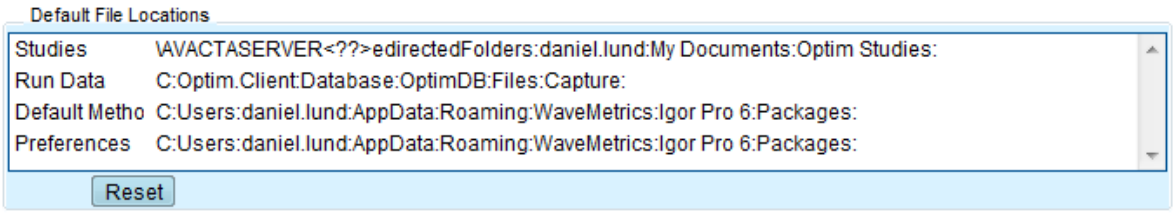


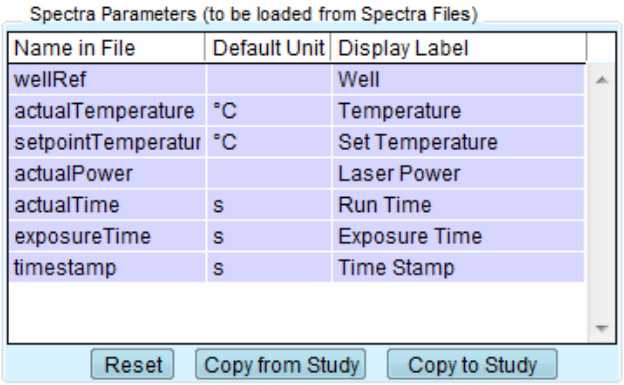
In LOAD DATA settings the paths to the file locations can be configured (as mentioned in Advanced mode starting on page 66.



It is also possible to change the location where the default analysis methods and these preferences are saved.

As discussed on page 52, the spectra acquired by the Optim Client software contain headers with information about the properties of the instrument at the time the individual spectra were acquired. The information relates to the well from which the spectrum was acquired, the temperature of the sample at the time, the set temperature, the laser power measured, the time from the start of the experiment, the exposure time and the time and date that the spectrum was acquired. This information can be represented in the analysis system by a display label, and this can be set in the LOAD DATA settings.

Changing the Display label will change the labels through the analysis software, whether it is in method development, tables or graphs of results.



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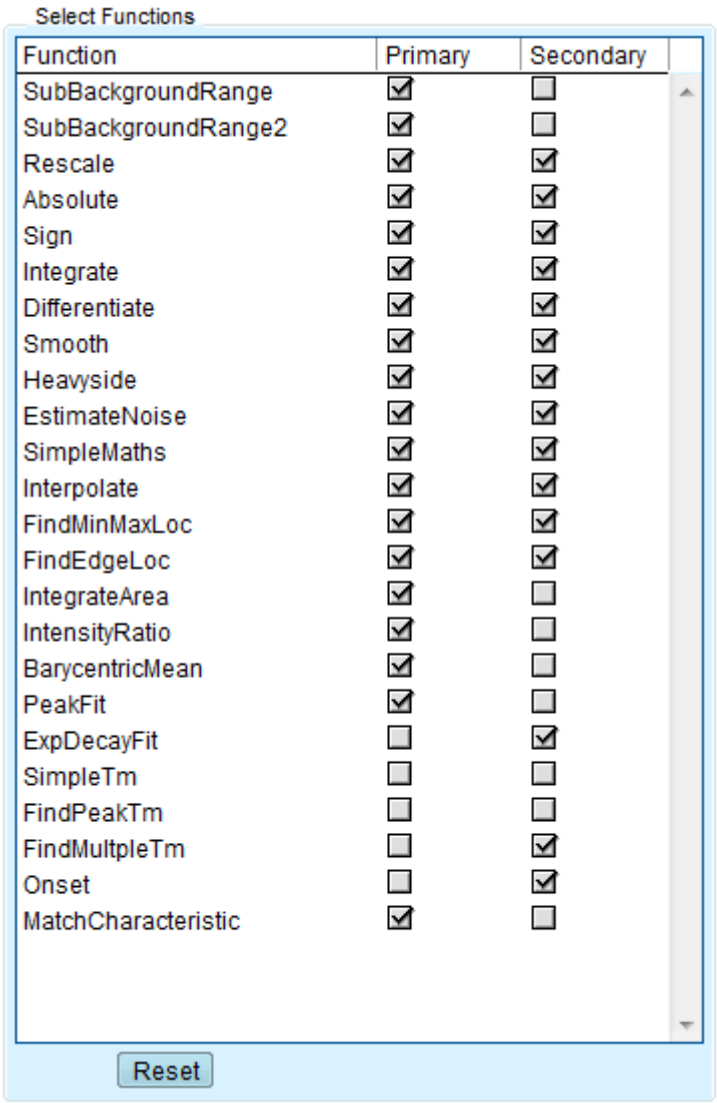
If you edit these settings, you can save them as defaults by clicking SAVE SETTINGS in the GENERAL settings. Clicking RESET will reset them to the original saved settings if you have changed then.

The ANALYSIS settings allow you to change which functions appear in the primary, secondary or tertiary analysis sections of the Method Developer. Checking the relevant column will make that function available for each analysis level.



Clicking RESET will reset the selections to the Avacta Analytical supplied options.

As discussed in the previous section, the instrument obtains different parameters that can be plotted against analysis results to show trends. By default, only the actual measured temperature and the time after the start of the experiment are available for display. If you wish to change the available parameters you can do so in the ANALYSIS settings.

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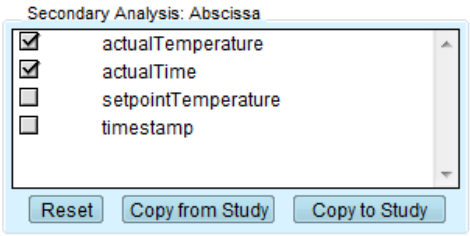
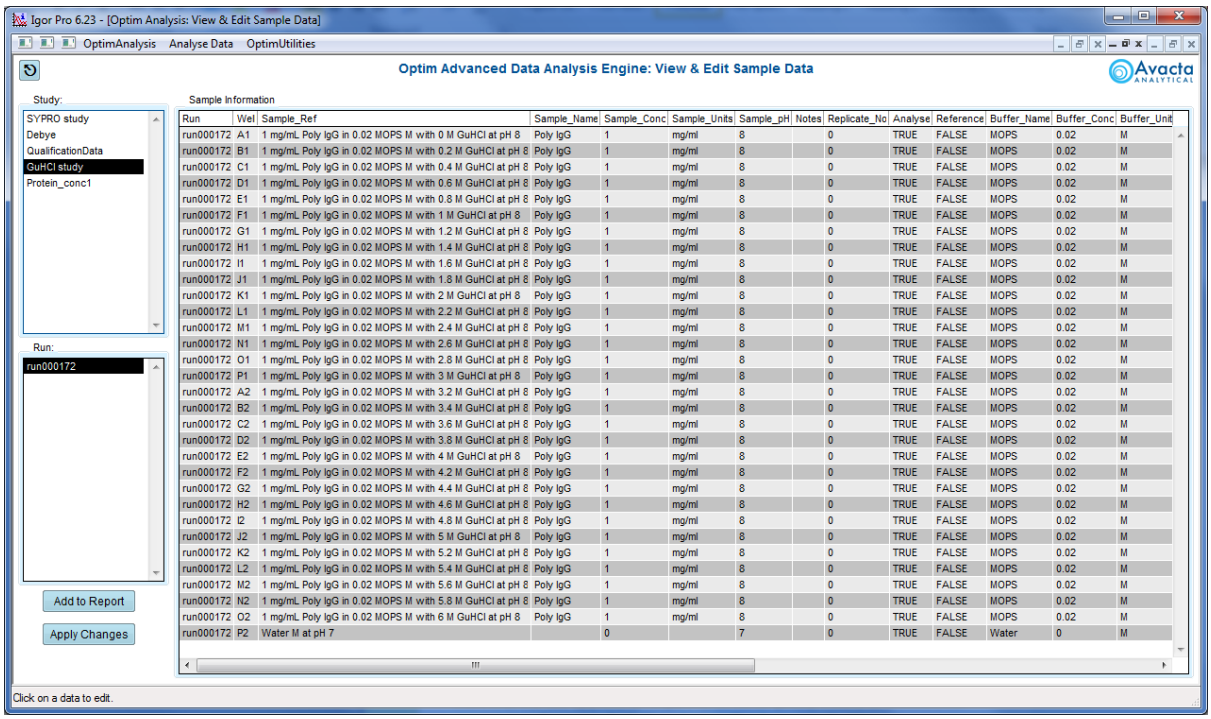


Before you start – adjusting/verifying the sample formulation

During the pre-run configuration in the client software information can be entered that defines the formulation conditions for each of the sample measured. If this information is not supplied or is entered incorrectly it can lead to confusion during analysis, or not being able to perform tertiary analysis.

It is possible to view and edit this information in the Optim Analyser software. Selecting the **Optim Utilities** menu and selecting *View and Edit sample data* will display a table view of this information that can be edited.



Run	Wel	Sample_Ref	Sample_Name	Sample_Conc	Sample_Units	Sample_pH	Notes	Replicate_No	Analyse	Reference	Buffer_Name	Buffer_Conc	Buffer_Unit
run000172	A1	1 mg/mL Poly IgG in 0.02 MOPS M with 0 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	B1	1 mg/mL Poly IgG in 0.02 MOPS M with 0.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	C1	1 mg/mL Poly IgG in 0.02 MOPS M with 0.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	D1	1 mg/mL Poly IgG in 0.02 MOPS M with 0.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	E1	1 mg/mL Poly IgG in 0.02 MOPS M with 0.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	F1	1 mg/mL Poly IgG in 0.02 MOPS M with 1 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	G1	1 mg/mL Poly IgG in 0.02 MOPS M with 1.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	H1	1 mg/mL Poly IgG in 0.02 MOPS M with 1.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	I1	1 mg/mL Poly IgG in 0.02 MOPS M with 1.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	J1	1 mg/mL Poly IgG in 0.02 MOPS M with 1.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	K1	1 mg/mL Poly IgG in 0.02 MOPS M with 2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	L1	1 mg/mL Poly IgG in 0.02 MOPS M with 2.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	M1	1 mg/mL Poly IgG in 0.02 MOPS M with 2.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	N1	1 mg/mL Poly IgG in 0.02 MOPS M with 2.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	O1	1 mg/mL Poly IgG in 0.02 MOPS M with 2.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	P1	1 mg/mL Poly IgG in 0.02 MOPS M with 3 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	A2	1 mg/mL Poly IgG in 0.02 MOPS M with 3.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	B2	1 mg/mL Poly IgG in 0.02 MOPS M with 3.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	C2	1 mg/mL Poly IgG in 0.02 MOPS M with 3.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	D2	1 mg/mL Poly IgG in 0.02 MOPS M with 3.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	E2	1 mg/mL Poly IgG in 0.02 MOPS M with 4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	F2	1 mg/mL Poly IgG in 0.02 MOPS M with 4.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	G2	1 mg/mL Poly IgG in 0.02 MOPS M with 4.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	H2	1 mg/mL Poly IgG in 0.02 MOPS M with 4.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	I2	1 mg/mL Poly IgG in 0.02 MOPS M with 4.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	J2	1 mg/mL Poly IgG in 0.02 MOPS M with 5 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	K2	1 mg/mL Poly IgG in 0.02 MOPS M with 5.2 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	L2	1 mg/mL Poly IgG in 0.02 MOPS M with 5.4 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	M2	1 mg/mL Poly IgG in 0.02 MOPS M with 5.6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	N2	1 mg/mL Poly IgG in 0.02 MOPS M with 5.8 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	O2	1 mg/mL Poly IgG in 0.02 MOPS M with 6 M GuHCl at pH 8	Poly IgG	1	mg/ml	8		0	TRUE	FALSE	MOPS	0.02	M
run000172	P2	Water M at pH 7		0		7		0	TRUE	FALSE	Water	0	M

Selecting a study and a run within that study will allow the information to be edited. In some cases, where there are only a defined number of options available, such as the "Analyse" column, clicking on a cell in this column will present a popup menu containing the available choices.

Changes made here need to be applied, so click APPLY CHANGES to save these changes back into the study. ADD TO REPORT will add the information to the sample report as a series of tables containing related information that are formatted to fit into the width of the page.

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IMPORTANT Changing the sample information has a direct impact on the content of the default tertiary analysis collections. It is important to remake the default collections after changing the formulation information. Only if you have changed information in order to perform a buffer subtraction will you need to remake the primary analysis collections.

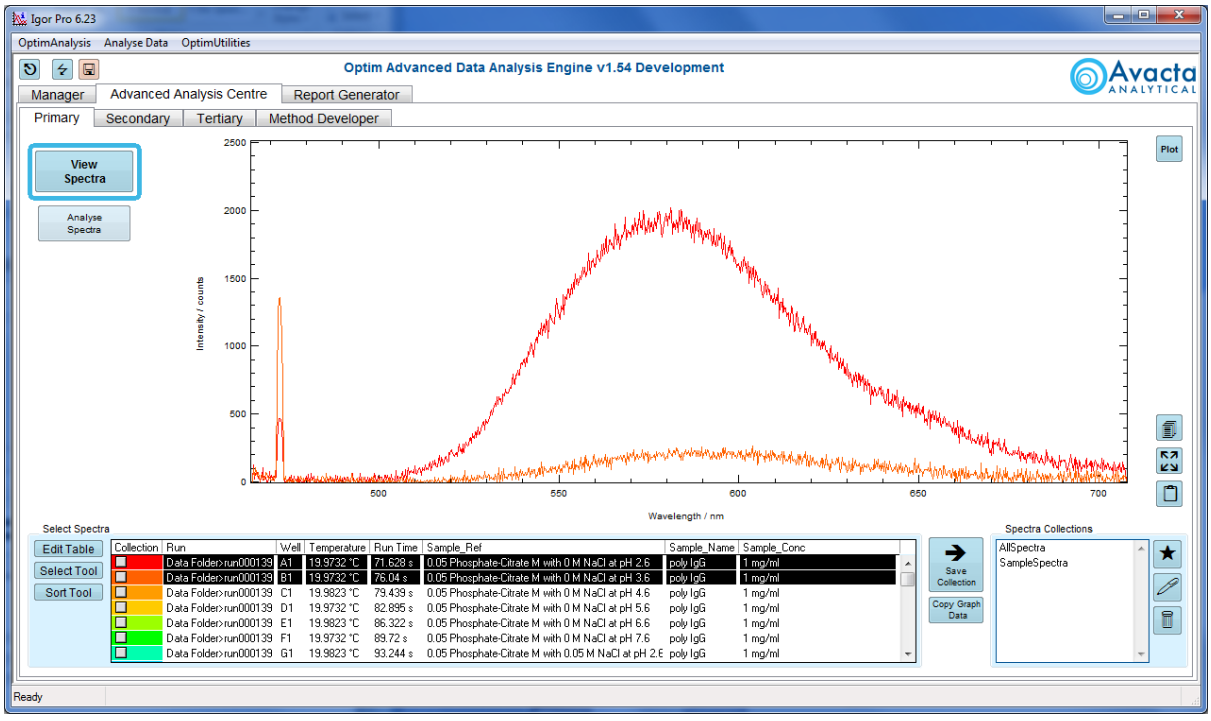
Primary Analysis

Primary analysis is the second phase of the data analysis process, after data loading. Analysis is completed by selecting a number of spectra, forming them into collections and then applying an analysis method to a selected collection. These collections can be either, groups of related spectra (so that different analysis methods can be applied as required) or, for simplicity's sake, one collection containing all spectra to which all analysis methods are applied.

The next section describes how to create spectra collections, how to create an analysis program and how to apply it to the spectra collections.

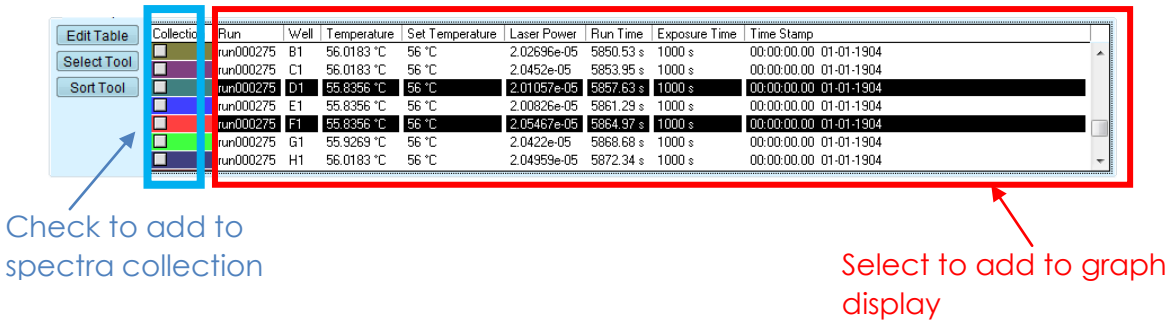
Looking at spectra data

Now that data has been loaded, the spectra can be sorted into collections. First, go to **Advanced Analysis Centre** tab and the **Primary** sub-tab and ensure that VIEW SPECTRA is selected.



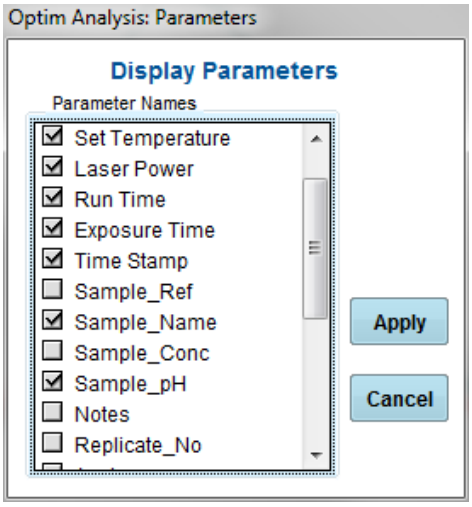
To display spectra:

- In the region at the bottom of the screen entitled Select Spectra select an individual spectrum by clicking on the text (not the checkbox).
- More spectra can be displayed by holding the shift key and clicking another row (or rows).



There are two clickable regions in the list of spectra. By checking a box in the column entitled "Collection", the spectrum can be added to a spectra collection. By clicking on the text in the row, the spectrum is displayed in the graph. The colour of the background in the collection column matches with the colour of the trace in the graph.

- EDIT TABLE – clicking this will allow you to select which parameters relating to the samples are displayed in the table, such as the well reference, the temperature, the sample name, pH etc. Simply check the field you wish to display then click APPLY.



- SELECT TOOL – this tool will enable you to select spectra to display or to prepare to add to a spectra collection for analysis.
    - Choose to group the spectra by the well reference, temperature, sample name, pH etc, then select the value you wish in the right hand list.
    - Choose whether to add the selected spectra to the displayed graph (check *Graph*), a spectra collection (check *Collection*) or both.
    - Select whether to add or remove the selection to or from your displayed graph or spectra collection by selecting ADD or REMOVE respectively. To start a new graph or spectra collection click NEW. In the case of the example below, all spectra with a set temperature of 18 °C will be selected.

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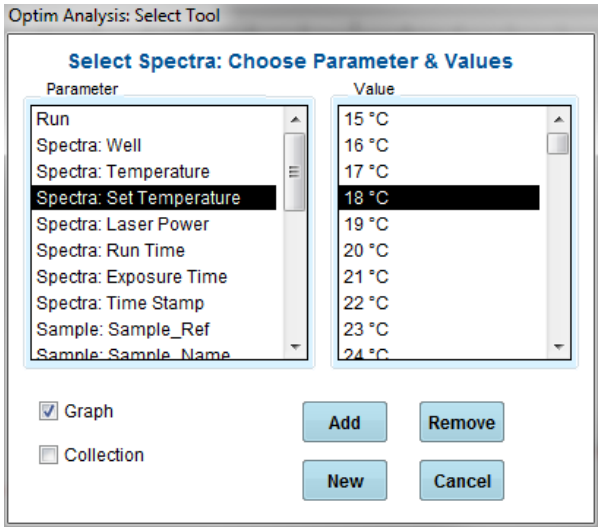
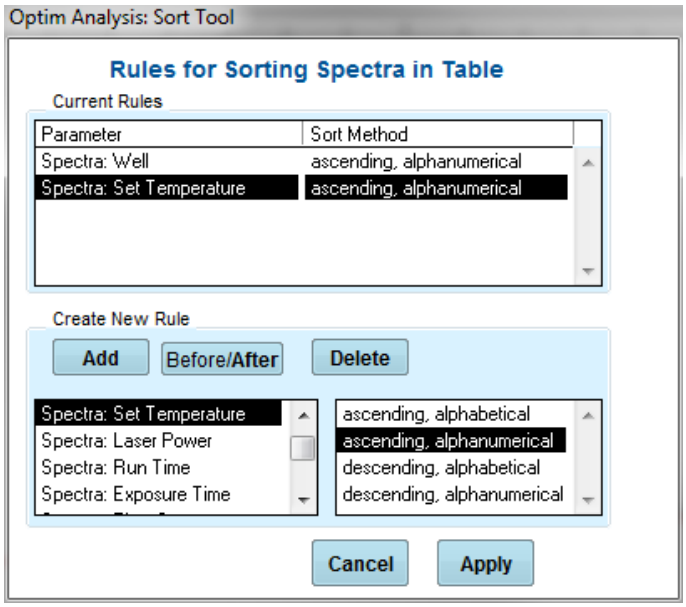


- SORT TOOL – a tool to allow sorting of spectra by any of the parameters in the table
    - Under Create New Rule, select which parameter you wish to sort the spectra list by, e.g., set temperature, well reference etc.
    - On the right, select how you wish to sort the spectra.
    - Click ADD to add this new rule to the current rules.
    - To add the new rule before or after the selected rule in the Current Rules click BEFORE/AFTER. The word in bold represents the current choice.
    - To delete a rule from the Current Rules, first select it then click DELETE.
    - To apply the sorting, click APPLY.
    - The example below will sort the spectra list by ascending alphanumerical well reference (all A1 well spectra will be shown first, then all A2 spectra etc), followed by ascending alphanumerical set temperature. (All A1 spectra will be displayed first in increasing temperature order, followed by A2 spectra in ascending temperature order, etc.).



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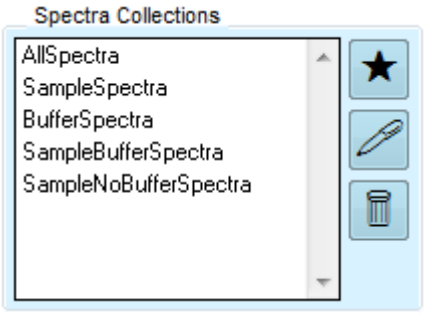
Once you have chosen and checked the spectra to analyse, click ➔SAVE COLLECTION. This will allow you to create a spectra collection with a name of your choice. It will then be displayed in the "Spectra Collections" list.



You can rename a collection by highlighting it in the list and clicking ✎ or delete one by clicking 🗑. The ★ will generate the default spectra collections.

The default collections are:

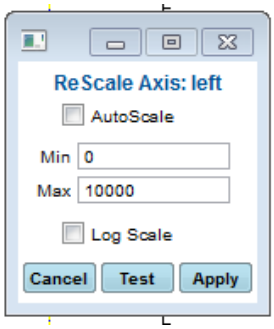
- **AllSpectra** – contains all spectra
- **BufferSpectra** – contains all spectra with REFERENCE set to TRUE
- **SampleSpectra** – contains all spectra with REFERENCE set to FALSE and ANALYSE set to TRUE
- **SampleBufferSpectra** – contains all spectra in SampleSpectra for which the software can find a buffer spectrum to subtract
- **SampleNoBufferSpectra** – contains all spectra in SampleSpectra for which the software cannot find a buffer spectrum to subtract

Clicking on COPY GRAPH DATA will copy the data in the graph to the clipboard such that it can be pasted into other software.

Clicking on 🗐 will add the graph on the screen to the currently open report. This is discussed in more detail later in this handbook.

Clicking on 📋 will copy the graph as an image and you can paste this into any software of your choice.

Right clicking on each axis will allow the scale to be changed. Ticking "AutoScale" will automatically set the scale of the graph. If this isn't ticked you can enter values for Min and Max as required. Ticking "Log Scale" will display the axis as a $\log_{10}$ plot.



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Before setting the scale, TEST will allow you to review the display before clicking APPLY to set the scale to the required values.

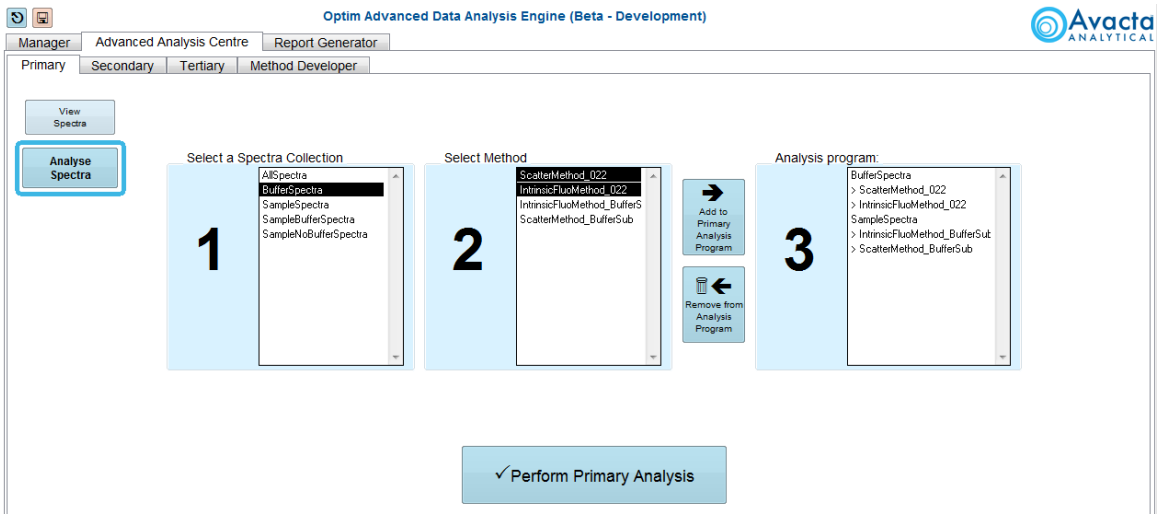
The button will autoscale the axes of the spectrum graph.

You can view the raw data as acquired on the CCD detector by clicking PLOT and selecting *Raw*. Selecting *Spectrum* from this list will return to the normal calibrated spectrum view.

Performing primary analysis on spectra lists

This section describes how to analyse the spectra collections using default analysis methods. Creating and editing analysis methods is covered in a later section.

Go to **Advanced Analysis Centre** tab and the **Primary** sub-tab and ensure that ANALYSE SPECTRA is selected.



Performing primary analysis is simple in the advanced analysis mode. A program is built up by the following process:

- Select a collection.
- Select a method to use to analyse.
- Click ➔ADD TO PRIMARY ANALYSIS PROGRAM.
- Repeat this process until you have built up your program.
- If you make a mistake, select the line in the Analysis Program that is wrong and click 🗑←REMOVE FROM ANALYSIS PROGRAM.
- When you have completed building up your program, click ✓PERFORM PRIMARY ANALYSIS.

The example shown above creates an analysis program that will look at the light scattering and intrinsic fluorescence profiles of buffer spectra in order to determine what effect temperature has on their spectra, and analyse the spectra by first subtracting a relevant buffer from each sample before calculating light scattering and intrinsic fluorescence characteristics.

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The default primary analysis methods consist of a method to analyse the static light scattering intensity called "ScatterMethod" and a method to analyse characteristics of the intrinsic fluorescence, "IntrinsicFluoMethod".

- Intrinsic Fluorescence
  - Calculates the **integrated intensity** of each spectrum in the wavelength range 280 – 460 nm. (The result of which is called FluoIntensity).
  - Determines an **intensity ratio** between the spectral intensity at 350 nm to that at 330 nm (called Ratio350_330).
  - Evaluates the expectation wavelength/**barycentric mean** of the spectra in the range of 280 – 460 nm. The barycentric mean is effectively the centre of mass (or a weighted average) of the fluorescence emission, and is given by $\lambda_m = \sum_\lambda f_\lambda \lambda / \sum_\lambda f_\lambda$ (called BarycenFluo).
  - Determines a **peak position** and a **peak height** by fitting a Lorentzian function to the fluorescence, initially centred at 340 nm with a width of 20 points and performing five iterations (called FluoPeakPos and FluoPeakHeight respectively).
- Scattering Intensity
  - Calculates the integrated intensity over the wavelength range 261 – 271 nm (called Scatter266).
  - Calculates the integrated intensity over the range 468 – 478 nm (called Scatter473).

There are also two further methods which are called "ScatterMethod_BufferSub" and "IntrinsicFluoMethod_BufferSub". These provide the same functionality as the other two methods but include an additional step where the software will match the provided characteristic information about individual sample wells to find an appropriate spectrum to subtract. For this to function correctly you need to have:

- Entered identical formulation information in the well in which the buffer will be loaded, but leave the Sample Details column empty.
- Changed the reference column entry to TRUE.

This will generate output results which have an appended "_B". For example, Ratio350_330 will be called Ratio350_330_B when a buffer spectrum has been subtracted.

Secondary Analysis

As with primary analysis, secondary analysis is performed by creating collections of inputs (in this case primary analysis results (unfolding curves and SLS curves)) and applying analysis methods. Secondary analysis looks at the time and/or temperature dependence of the fluorescence or light scattering results to obtain melting temperatures, aggregation onset temperatures and kinetic rate constant.

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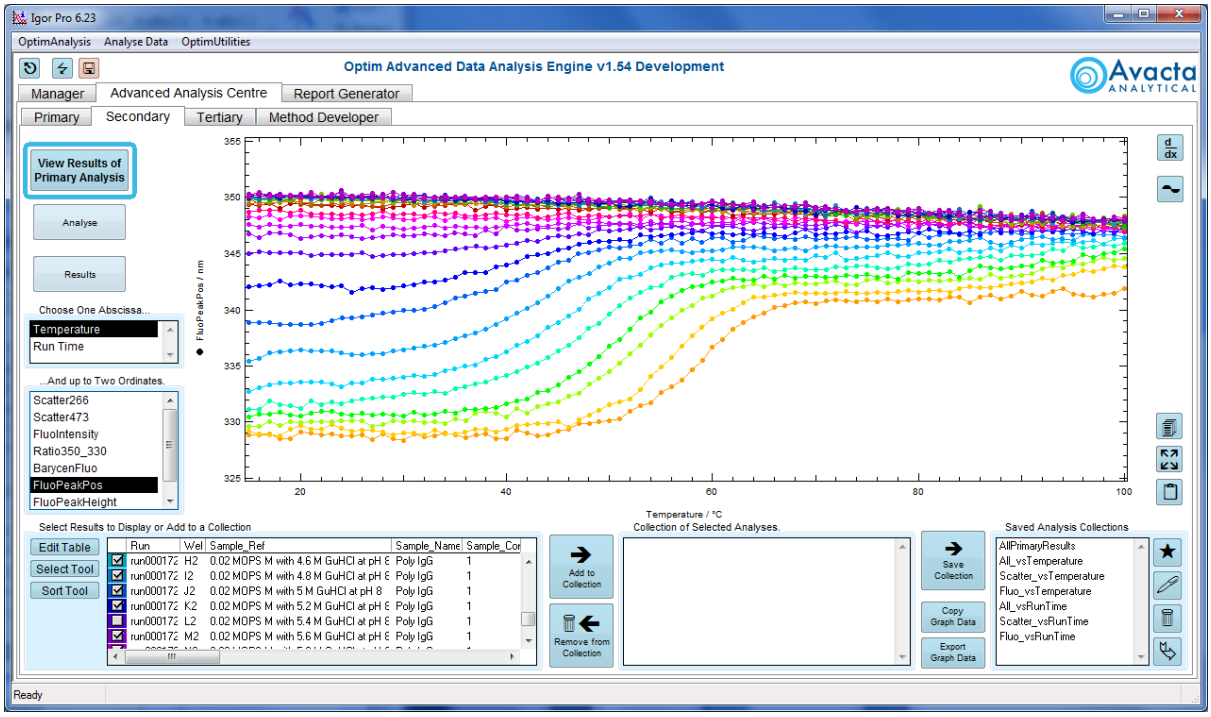

Looking at the curves and creating analysis collections

First go to **Advanced Analysis Centre** tab and the **Secondary** sub-tab and ensure that VIEW RESULTS OF PRIMARY ANALYSIS is selected.

Analysis collections are created by displaying the data that you wish to analyse. As with the primary analysis, it is possible to create a default list to analyse by clicking ★ in the Saved Analysis Collections – this will create a collection consisting of all the primary analysis results curves created against all the available secondary analysis abscissas – temperature and run time.

The default collections in this case are:

- **AllSecondaryData** – contains all the primary analysis results sorted against all abscissas.
- **All_vsTime** – contains all the primary analysis results sorted against run time (time zero is the start of the experiment).
- **Scatter_vsTime** – contains the Scatter266 and Scatter473 (and any buffer subtracted results) sorted against run time.
- **Fluo_vsTime** – contains the Scatter266 and Scatter473 (and any buffer subtracted results) sorted against run time.
- **All_vsTemp** – contains all the primary analysis results sorted against temperature.
- **Scatter_vsTime** – contains the Scatter266 and Scatter473 (and any buffer subtracted results) sorted against temperature.
- **Fluo_vsTime** – contains the Scatter266 and Scatter473 (and any buffer subtracted results) sorted against temperature.

To create a custom analysis collection:

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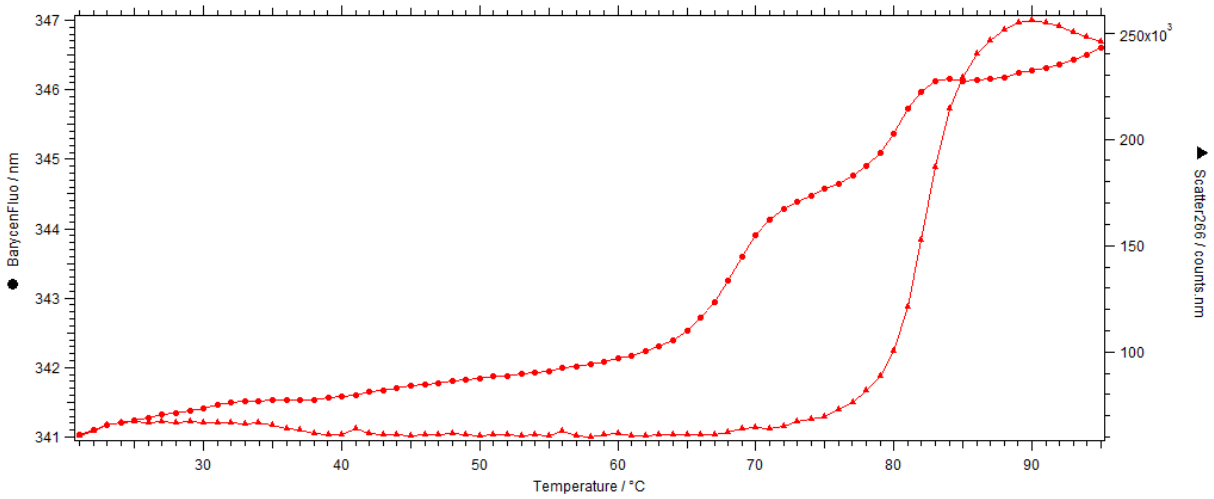
- Create the graph of the data you wish to add to the analysis collection
    - Select an abscissa (typically Temperature for a thermal unfolding experiment or Run Time for an isothermal experiment).
    - Select an ordinate (Scatter266 and Scatter473 for SLS data at 266 nm and 473 nm respectively, and the other parameters for fluorescence).
    - Choose samples from the list under "Select Results to Display or Add to a Collection". This will display curves in the plot display area, as you have selected.
- Click ➔ADD TO COLLECTION.
- Repeat until the collection contains all the information you wish.
- Data can be removed from the analysis collection by clicking 🗑REMOVE FROM COLLECTION.
- Click ➔SAVE COLLECTION.

As in the primary analysis section, you can use the EDIT TABLE, SELECT TOOL and SORT TOOL functionality to assist in the making of graphs.

It is sometimes useful to compare complementary datasets on a single plot. You can select up to two ordinates (shift click for selecting multiple ordinates) and an infinite number of sample curves. When you do so, the values for the second ordinate will be displayed on the right hand axis. The colour of traces for each well will be the same so they can be distinguished by the symbol of their marker – the left hand axis is a • (in this case representing the barycentric mean of fluorescence) and the right hand axis is a ▲ (in this case the static light scattered intensity at 266 nm).



In an example presented here, the analysis collection is created to contain the data from run000005 of the barycentric mean fluorescence and the scattered light intensity at 266 against temperature. This is then saved as an analysis collection called "MyAnalysisCollection".

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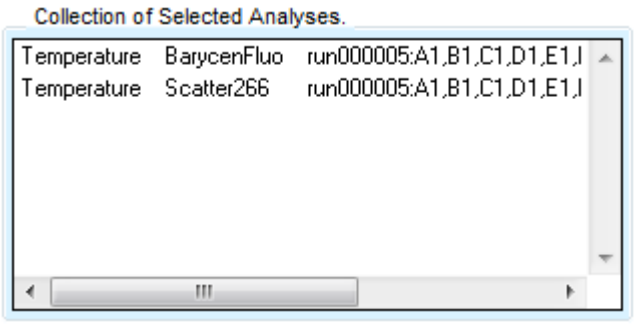


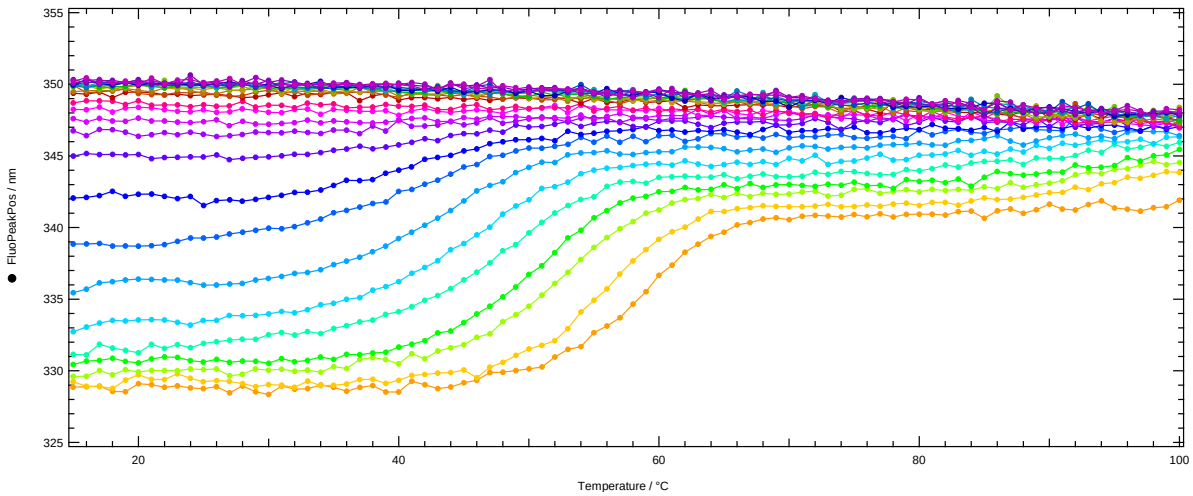
As before, the selected collection can be renamed by clicking ✎, deleted by clicking 🗑, and now exported to a .csv file by clicking ↳. All exported data is saved to the study folder in the Studies location (defined at the start of this handbook) with a filename of ordinate_vs_abscissa.csv. If a file of this description already exists, the new filenames will have a number appended sequentially with each export.

Clicking 🗐 will copy the displayed graph to the currently open report. This is discussed later in this handbook.

The data in the graph can be quickly extracted for use in other programs by clicking COPY GRAPH DATA. EXPORT GRAPH DATA will create a .csv file in the study folder with a filename comprised of the ordinate names and the abscissa. If a file of this description already exists, the new filenames will have a number appended sequentially with each export.

Changing the view of the data for a clearer picture

The button labelled ◓ will allow you to smooth your data. The data shown below is unsmoothed.



Despite this it is still easy to see the different trends in the samples (in this case these samples are protein that has increasing concentrations of GuHCl). Smoothing the data can make the graphs look smoother (hmm…).

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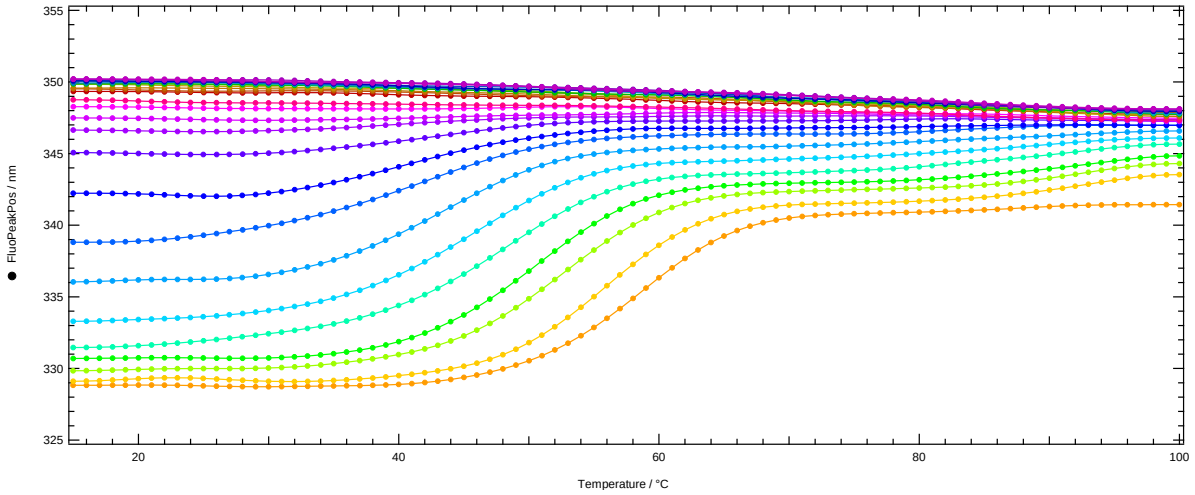
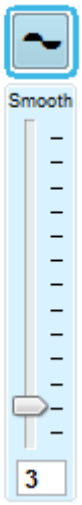


Selecting one of these will display a slider such that the width of the smooth can be selected.



The larger the smooth width, the lower the resolution of the curves and there is a potential to artificially remove smaller, less significant transitions from the data. If in doubt, a good default choice is a 3-point binomial smooth – this will present the data well without introducing significant artefacts.

Clicking on d/dx will present the data as a differential of the unfolding curves. There are options to display the differential before it is smoothed, or after it is smoothed. Displaying the differential unsmoothed is not recommended as this is always noisy due to small fluctuations in the signal being amplified in a differential view. The top graph below represents an unsmoothed differential and the bottom graph has a three point (the minimum possible) smoothing applied.

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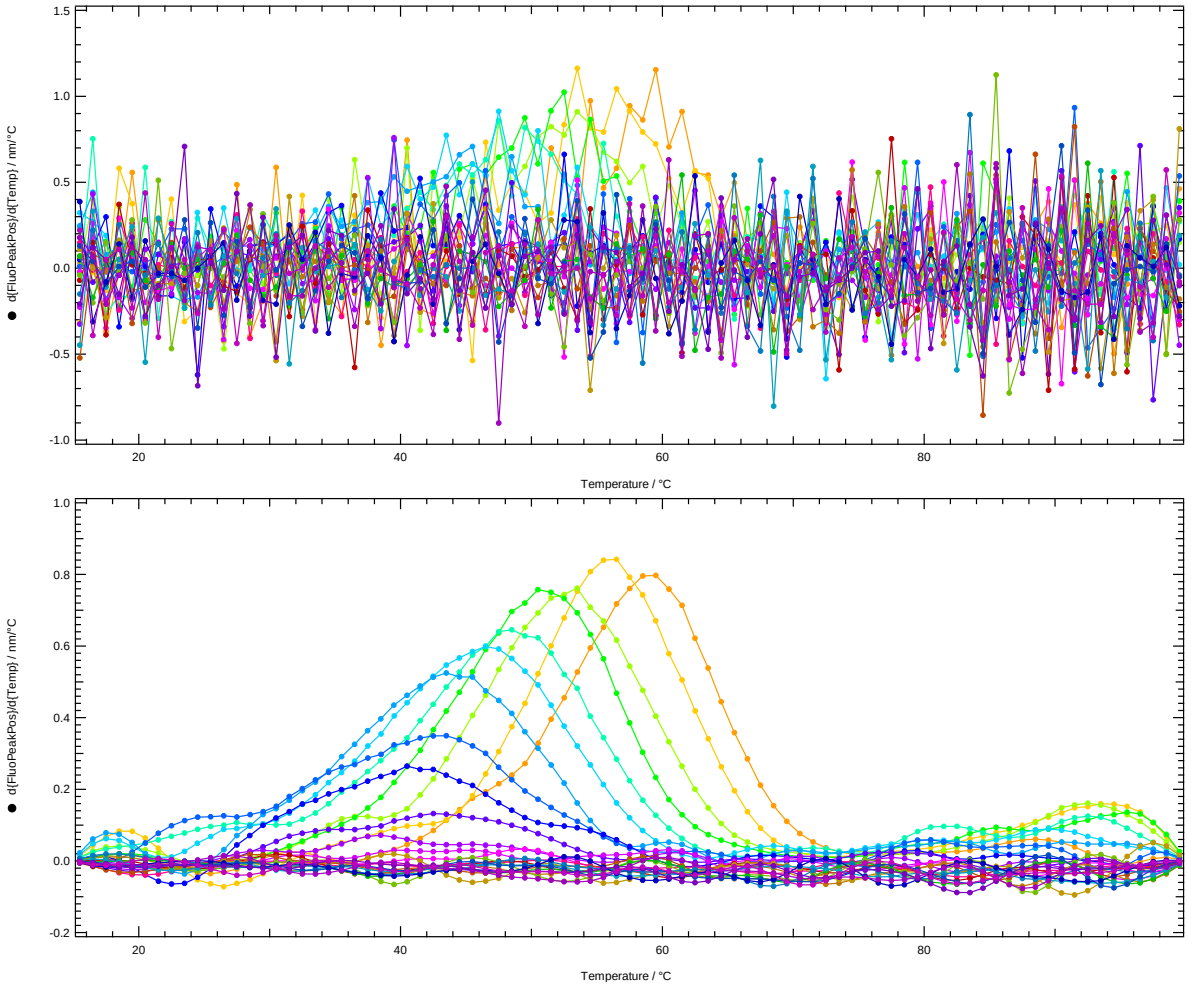
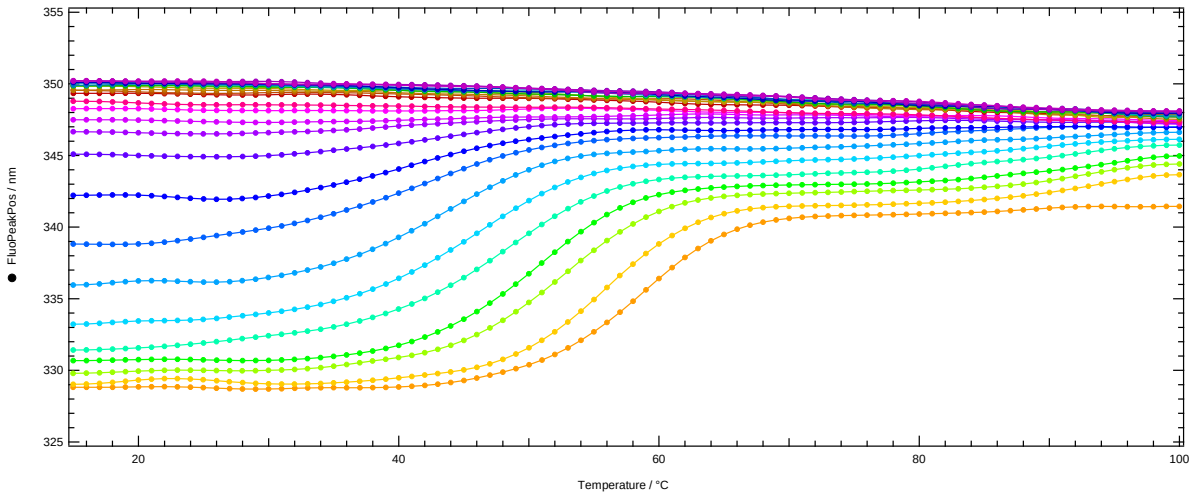


This data is the same as displayed above and as such the difference in the way the data is presented is clear (repeated below).



The advantage of displaying differentiated data is that it is sometimes clearer to the observer where the transition temperatures are, due to them being at the position of a peak maximum rather than the steepest point in a curve. However, by displaying the data in this way important information about tertiary structure (indicated by the start and end points of the undifferentiated curves) is lost.

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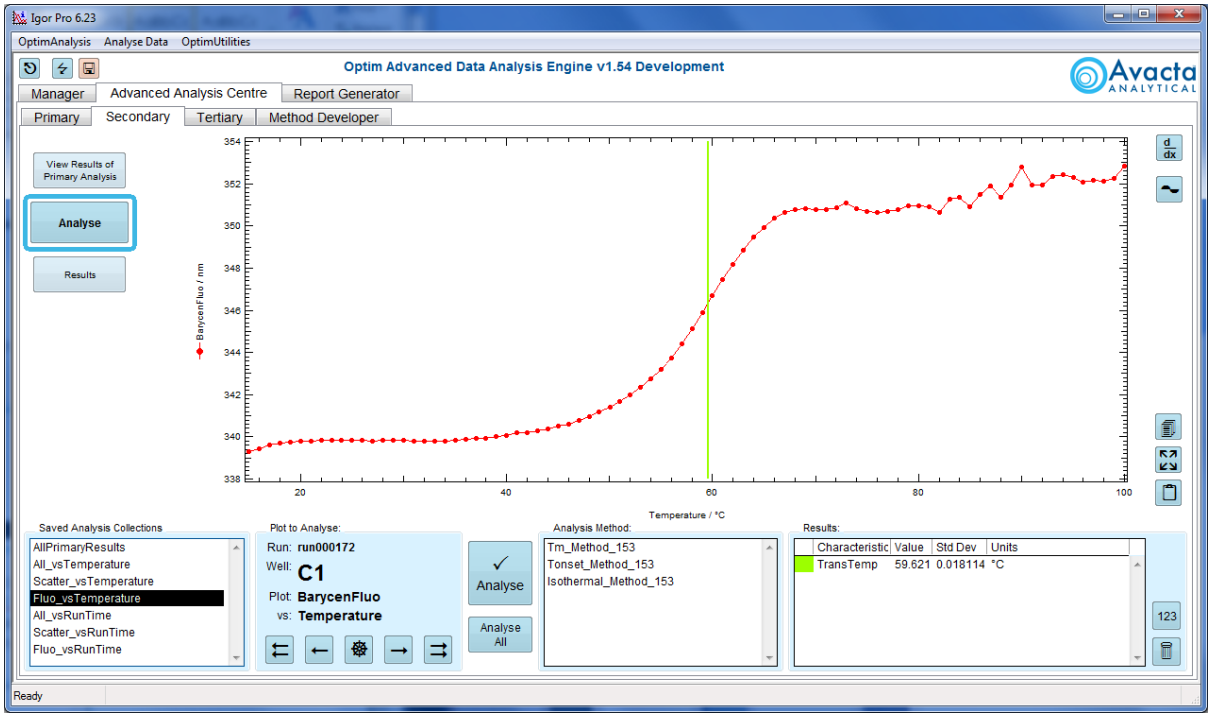
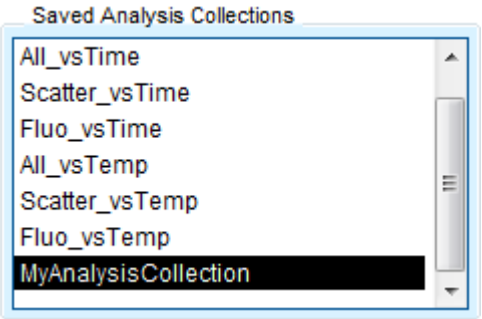
Performing the secondary analysis

Go to **Advanced Analysis Centre** tab and the **Secondary** sub-tab and ensure that ANALYSE is selected.

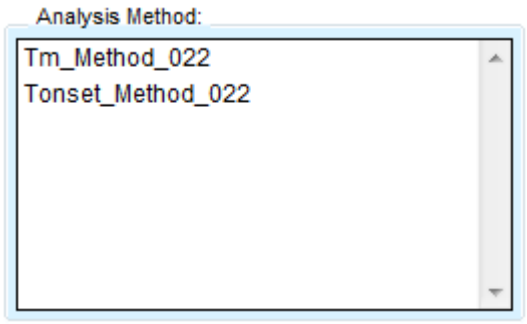


To analyse this data:

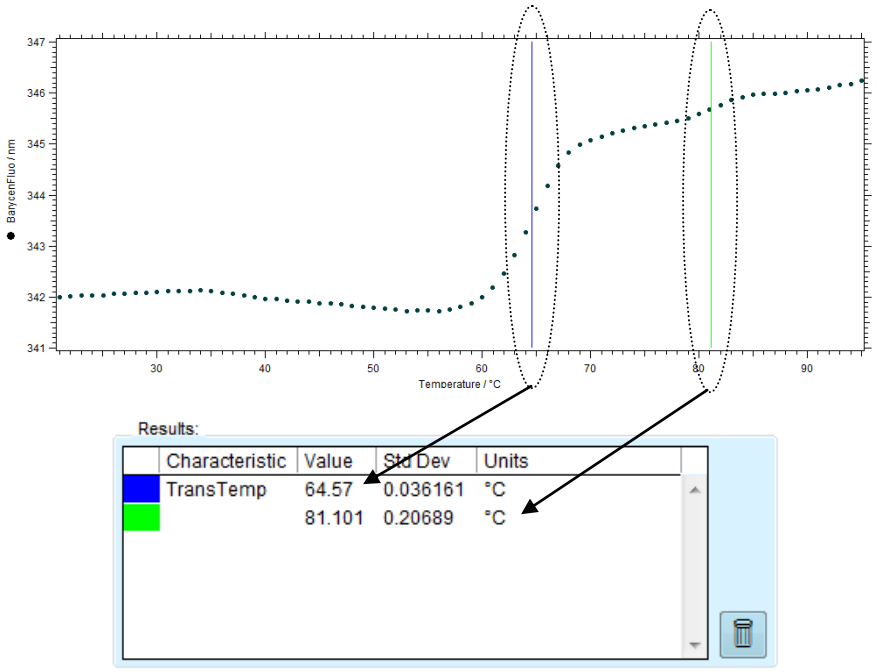
- Select the Analysis Collection that you wish to analyse – in this case MyAnalysisCollection is selected.



- Select the methods you wish to use – typically select Tm_Method for fluorescence data vs temperature, Tonset_Method for scattering data vs temperature (and fluorescence data to get the onset of unfolding) and Isothermal_Method for collections which include a variation with time.



- To analyse the trace on screen click ✓ANALYSE.
- View the result as a line on the graph and match the colour with the value displayed in the Results box.



- If the method has resulted in an error in the calculation you can select the relevant result by clicking on it in the graph, then:
    - Drag it to the location where you wish it to be, right click and select *Set*.
    - To reset to the calculated value select *Reset*.
    - To remove the result altogether (if a false positive) select *Remove*.
- If you wish to add a result at a particular point, hover over it with the pointer, right click and select *Add Result*. From the following menu select the type of result you wish to add. In order to add a result of a particular type you need to analyse the saved analysis collection you are looking at with the relevant method first.
- To remove all of the analysis results, click 🗑.
- You can remove results in the table by right clicking and selecting *Remove*.
- You can change the order of the results (potentially important for tertiary analysis) by clicking the button "123" (normally the transitions are ordered on significance).

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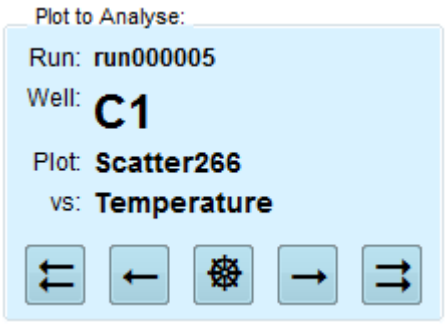
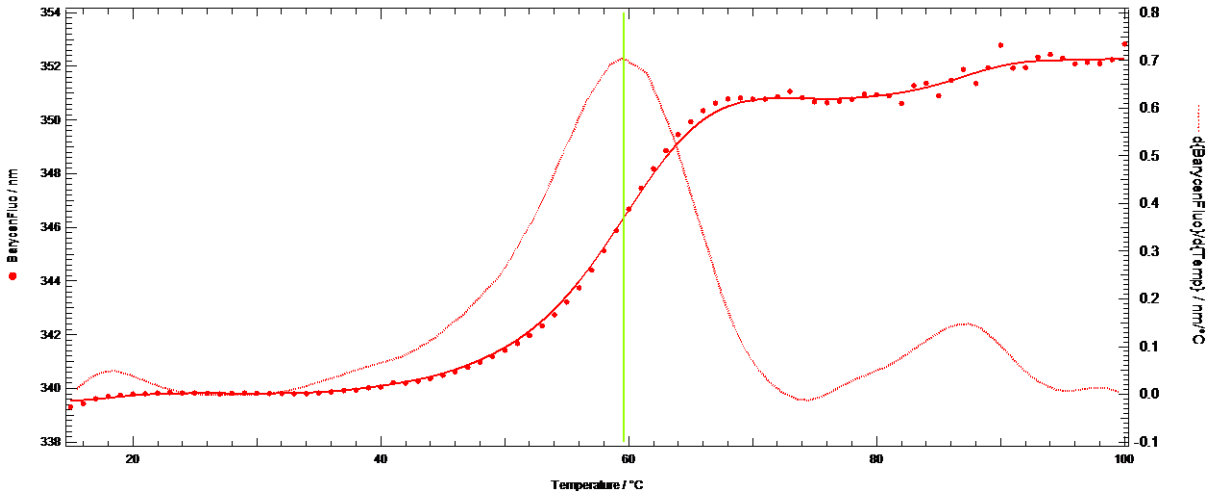
The default method will analyse the contents of the analysis collection by well, ordinate and abscissa. The section entitled "Plot to Analyse" displays the data that will be analysed. In the case of the example presented above, well D1 is presented from run000005, with an ordinate of barycentric mean fluorescence and an abscissa of temperature.



To analyse the rest of the data you can:

- Continue to analyse them individually
  - Cycle through each well by clicking → to move to the next well and ← to move to the previous well.
  - Cycle through each ordinate by clicking ⇉ to move to the next ordinate and ⇇ to move to the previous ordinate.
  - Click ✓ANALYSE at each graph with the respective method selected.
  - Clicking ❁ will reset the view to the first well, first ordinate and first abscissa.
- Analyse all data in an analysis collection using a selected method by clicking ANALYSE ALL.

It is sometimes easier, as already discussed in the previous section, to see transition temperatures in data that has been smoothed or differentiated. Showing the smoothed and differentiated curves in this case overlays onto the normal graph and allows a clear determination of transition temperatures.



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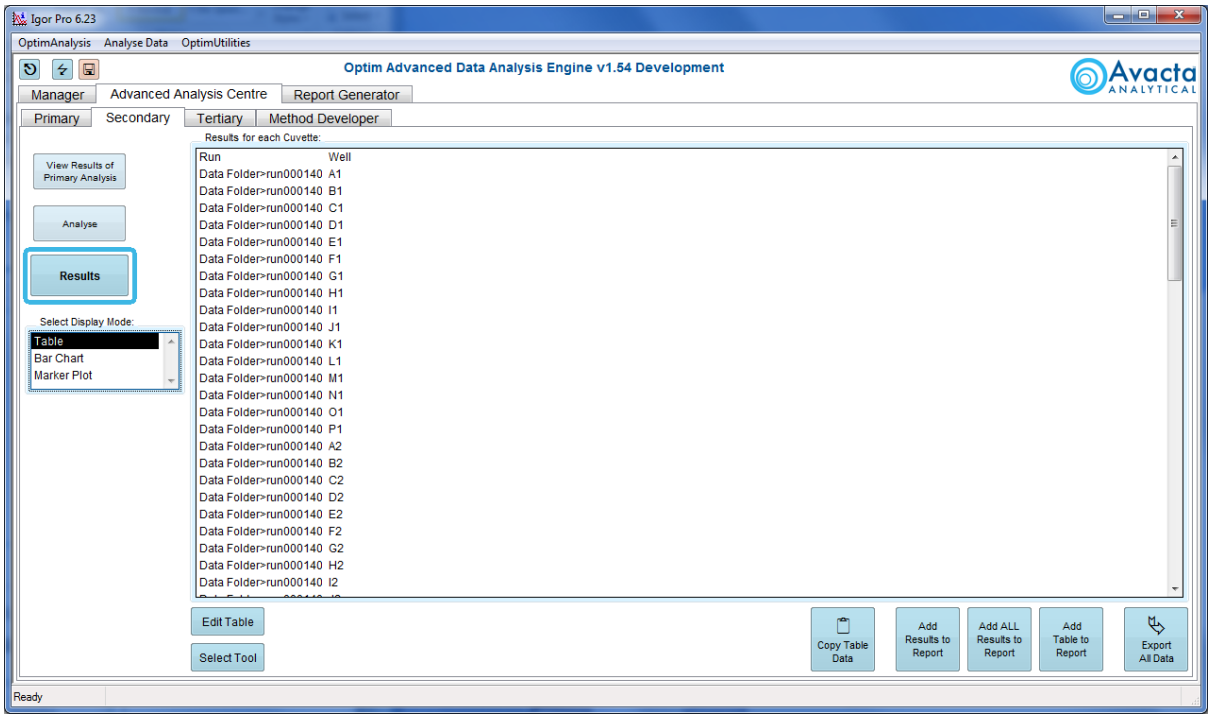
As with all graphs in the advanced analysis, the usual options to add the graph to the report, right clicking on axes to scale, and copying the graph to the clipboard apply.

The default secondary analysis methods are (further details are available in the method development section of this handbook):

- **Tm_Method** – uses an interpolation and a multiple Tm finding algorithm to determine multiple melting points with a sensitivity that varies depending on the intrinsic noise level of the data. This gives an output TransTemp that contains multiple results sorted by significance. The method also returns an uncertainty.
- **Tonset_Method** – uses sophisticated edge detection algorithms to calculate the onset temperature of either unfolding or aggregation.
- **Isothermal_Method** – fits a mono-exponential curve to the data.

Viewing the table of results

Finally, go to **Advanced Analysis Centre** tab and the **Secondary** sub-tab and ensure that RESULTS is selected.



To change the data that is displayed click EDIT TABLE.

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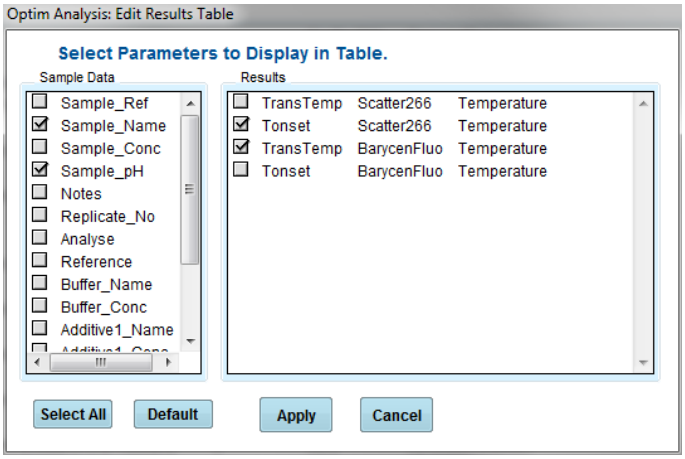

Sample data that is available to display can be checked, as can results of the secondary analysis. Clicking APPLY will close the edit table window and apply the settings to the results table.

Results for each Cuvette:

Run	Well	Sample_Name	Sample_pH	Scatter266 Temperature Tonset	BarycenFluo Temperature TransTemp
run000005	A1	1 mg/ml MabThera in 0 M Histidine	0	69.5	67.846±0.13001
					79.769±0.4381
run000005	B1	1 mg/ml Avastin in 0 M Histidine	0	68.5	69.283±0.061182
					80.359±0.23853
run000005	C1	1 mg/ml Herceptin in 0 M Histidine	0	77.5	42.997±0.48634
					68.382±0.088749
					79.985±0.18559
run000005	D1	1 mg/ml Remicade in 0 M Histidine	0	58.5	64.57±0.036161
					81.101±0.20689
run000005	E1	1 mg/ml Synagis in 0 M Histidine	0	75.5	67.962±0.096526
					80.633±0.088372
run000005	F1	1 mg/ml Humira in 0 M Histidine	0	69.5	69.748±0.14202
					80.902±0.065845
run000005	G1	1 mg/ml Enbrel in 0 M Histidine	0	67.5	63.722±0.11221
					79.309±0.073107
run000005	H1	1 mg/ml Erbitux in 0 M Histidine	0	66.5	66.559±0.052084
					83.183±0.73959

This data can then be copied to the clipboard by clicking COPY TABLE DATA.

The data can be exported to a comma separated variable file called SecondaryResults.csv by clicking on EXPORT ALL DATA.

The data can be added to the report by clicking:

- ADD RESULTS TO REPORT – will add the data to the report, but only those shown in the table, and it will add each result in a separate table.
- ADD ALL RESULTS TO REPORT – will add all of the results to the report, adding each result in a separate table.
- ADD TABLE TO REPORT – will add the data exactly as shown in the table into the report.

The secondary analysis results can also be represented in graphical form. Selecting either *Bar Chart* or *Marker Plot* will display the results in this way. To display results select a plot parameter, such as TransTemp, Tonset etc, and a dataset.

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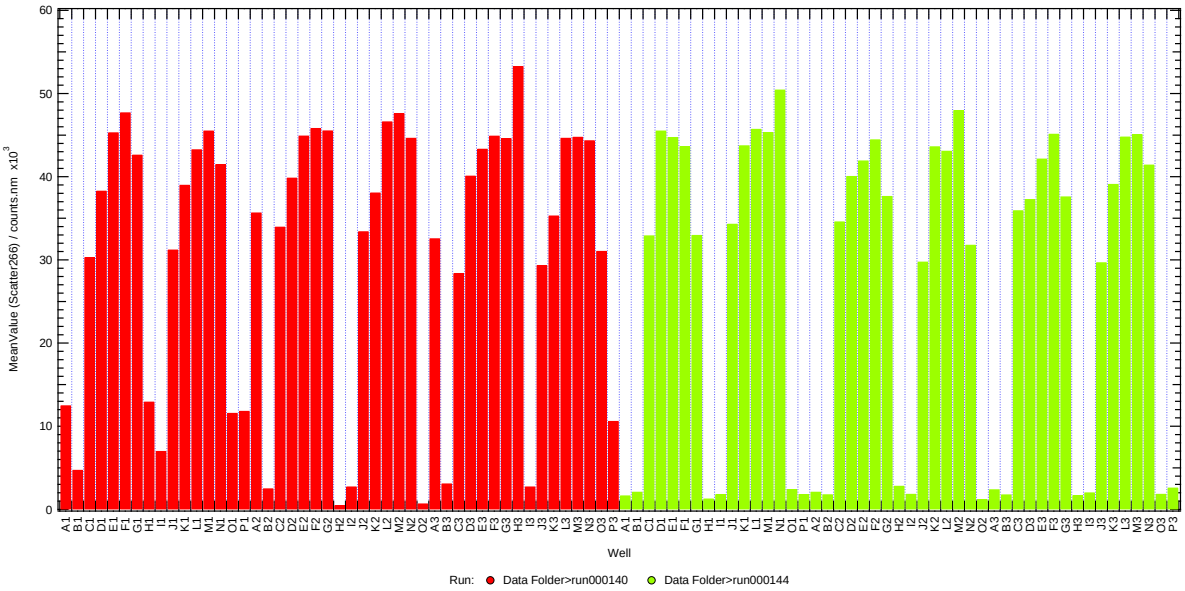
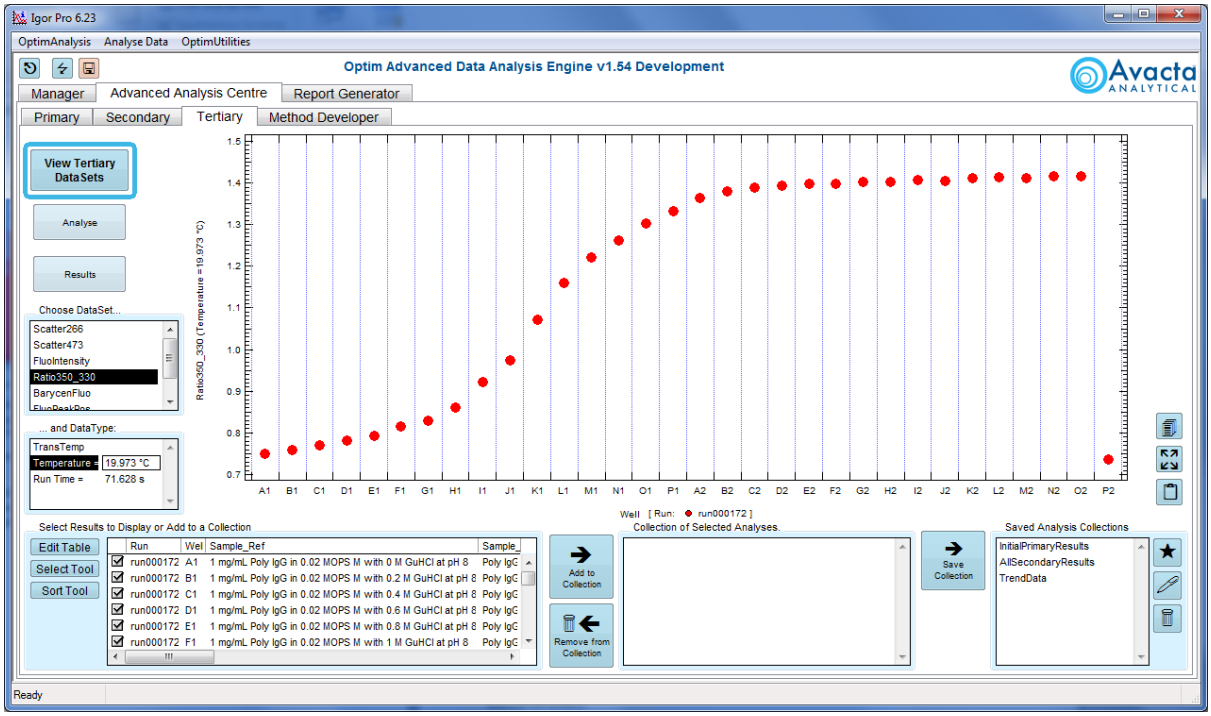


If there are multiple runs contained within the study and secondary analysis has been completed for both runs, each run will have a different colour that can be seen through the key at the bottom of the graph.

Right clicking on the graph will allow error bars to be shown or hidden.

Tertiary Analysis

Tertiary analysis looks at inter well variations in all parameters, such as primary analysis results (like peak position, fluorescence intensity *etc.*), secondary analysis results (transition temperatures, aggregation onset temperatures) and other special functions (such as Debye plots).


Igor Pro 6.23
OptimAnalysis Analyse Data OptimUtilities
Optim Advanced Data Analysis Engine v1.54 Development
Avacta ANALYTICAL
Manager Advanced Analysis Centre Report Generator
Primary Secondary Tertiary Method Developer
View Tertiary DataSets
Analyse
Results
Choose DataSet...
Scatter266
Scatter473
FluoIntensity
Ratio350_330
BarycenFluo
... and DataType:
TransTemp
Temperature = 19.973 °C
Run Time = 71.628 s
Ratio350_330 (Temperature = 19.973 °C)
Well [Run: run000172]
Select Results to Display or Add to a Collection
Edit Table
Select Tool
Sort Tool
Run Wel Sample_Ref Sample_
run000172 A1 1 mg/mL Poly IgG in 0.02 MOPS M with 0 M GuHCl at pH 8 Poly IgG
run000172 B1 1 mg/mL Poly IgG in 0.02 MOPS M with 0.2 M GuHCl at pH 8 Poly IgG
run000172 C1 1 mg/mL Poly IgG in 0.02 MOPS M with 0.4 M GuHCl at pH 8 Poly IgG
run000172 D1 1 mg/mL Poly IgG in 0.02 MOPS M with 0.6 M GuHCl at pH 8 Poly IgG
run000172 E1 1 mg/mL Poly IgG in 0.02 MOPS M with 0.8 M GuHCl at pH 8 Poly IgG
run000172 F1 1 mg/mL Poly IgG in 0.02 MOPS M with 1 M GuHCl at pH 8 Poly IgG
Add to Collection
Remove from Collection
Collection of Selected Analyses.
Save Collection
Saved Analysis Collections
InitialPrimaryResults
AllSecondaryResults
TrendData
Ready

There are three typical analyses that can be performed using tertiary analysis.

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- Averaging of replicates to determine reproducibility – either of any primary analysis result (such as the light scattering intensity at any measured temperature), or a secondary analysis result, like a Tm, rate constant or Tagg
- Trend analysis – looks at trending data and performs a mathematical fit to describe what is happening. For example
    - looking at how pH affects the Tm of a protein in solution (we will see some examples later)
    - fitting a thermodynamic two state model to some chemical unfolding data to obtain free energy change of unfolding or solvent accessible surface area
- Debye plots to give second virial coefficients and an estimate of molecular weight.

Looking at the data and creating analysis collections

First go to **Advanced Analysis Centre** tab and the **Tertiary** sub-tab and ensure that VIEW TERTIARY DATASETS is selected. At this point it is possible to display a selection of data (such as transition temperatures) that may have variation in solvent conditions/concentrations from well to well.

By selecting a dataset (these are the primary analysis results, including by default options such as Scatter266 for the 266 nm light scattering) a further range of "datatypes" become available. These data types include "slices" through the thermal unfolding/isothermal data (which will appear as options such as "Temperature = 15 C" and "RunTime = 60 s") and secondary analysis results (such as TransTemp and Rate Constant etc). Choosing combinations of these options and wells in the list box will display the graph on the screen – changing the temperature or run time will change the particular slice that is being displayed.

Clicking ➔ ADD TO COLLECTION will add the displayed data to the collection. Selecting items in the collection and clicking 🗑 ➔REMOVE FROM COLLECTION. Once you have selected all the items that you wish to add to your collection you can ➔ SAVE COLLECTION to take forward to the analysis.

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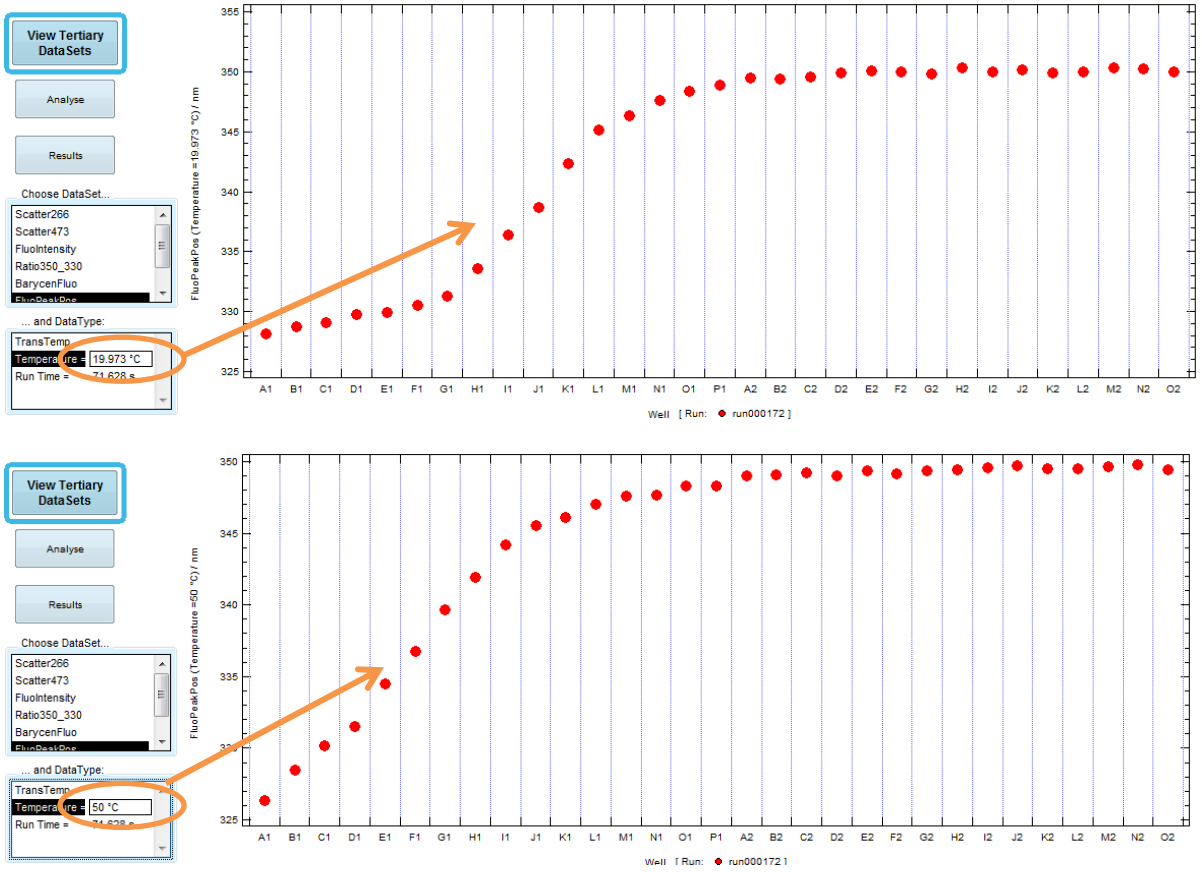

Clicking on ★ will create the default collections which will vary depending on the type of data that you have.

At this point, the Optim Analysis software will interrogate the sample information that was supplied as part of the experiment. If you haven't supplied complete information regarding the composition of the samples that you are measuring you can use the sample information editor that is accessible via clicking *View and Edit Sample Data* in the **Optim Utilities** menu.

The software will look for patterns in this information and group data accordingly. For example, if you have two types of samples:

- IgG in buffer 1 at pH 2 – 7
- IgG in buffer 2 at pH 2 – 7

then there will be two different sets of data to analyse for trends in primary slice data and secondary analysis results with pH.

The default collections that are formed are:

- **Initial Primary results** – this is a collection that contains the primary slice data that is acquired at the start of the experiment
- **All Secondary Results** – contains all datasets that have secondary analysis results, such as TransTemp etc
- **Replicates** – all datasets secondary analysis results and initial primary slice results for wells that have a non-zero replicate number defined in the sample

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information. If you have chosen replicates manually using this feature this will be where the data is collected

- **Duplicates** – all datasets with secondary analysis results or primary slice data but for wells that contain identical
    - Sample Name and concentration
    - pH
    - Buffer name and concentration
    - Additive names and concentrations
- **Debye Data** – contains all initial primary slice light scattering data, and any secondary analysis results with the same units (for example, time average data) where there is a variation in sample concentration. There must also be at least one well containing a reference (generally the buffer that the protein is dissolved in) that has zero concentration of the buffer protein present and all other solvent characteristics the same, and a solvent well containing just a single buffer and nothing else. (This solvent well may be the same as the reference depending on the particular formulation.)
- **Trend Data** – contains all data sets and data types that have a varying parameter. So, if there are samples with different concentrations of a particular excipient, but all other conditions are the same then they will be grouped together in the collection.

To create a custom analysis collection:

- Create the graph that you wish to add to the analysis collection
    - Select a dataset (which is a primary analysis result dataset, such as Scatter 266).
    - Select a datatype (Tonset, TransTemp, data at a particular temperature)
    - Choose samples from the list under "Select Results to Display or Add to a Collection". This will display curves in the plot display area, as you have selected
- Click ➔ ADD TO COLLECTION
- Repeat until the collection contains all the information you wish.
- Data can be removed from the collection by clicking 🗑 ➔REMOVE FROM COLLECTION
- Click ➔SAVE COLLECTION.

As in the primary and secondary analysis sections, you can use the EDIT TABLE, SELECT TOOL and SORT TOOL functionality to assist in the making of graphs.

Performing tertiary analysis

Go to **Advanced Analysis Centre** tab and the **Tertiary** sub-tab and ensure that ANALYSE is selected.

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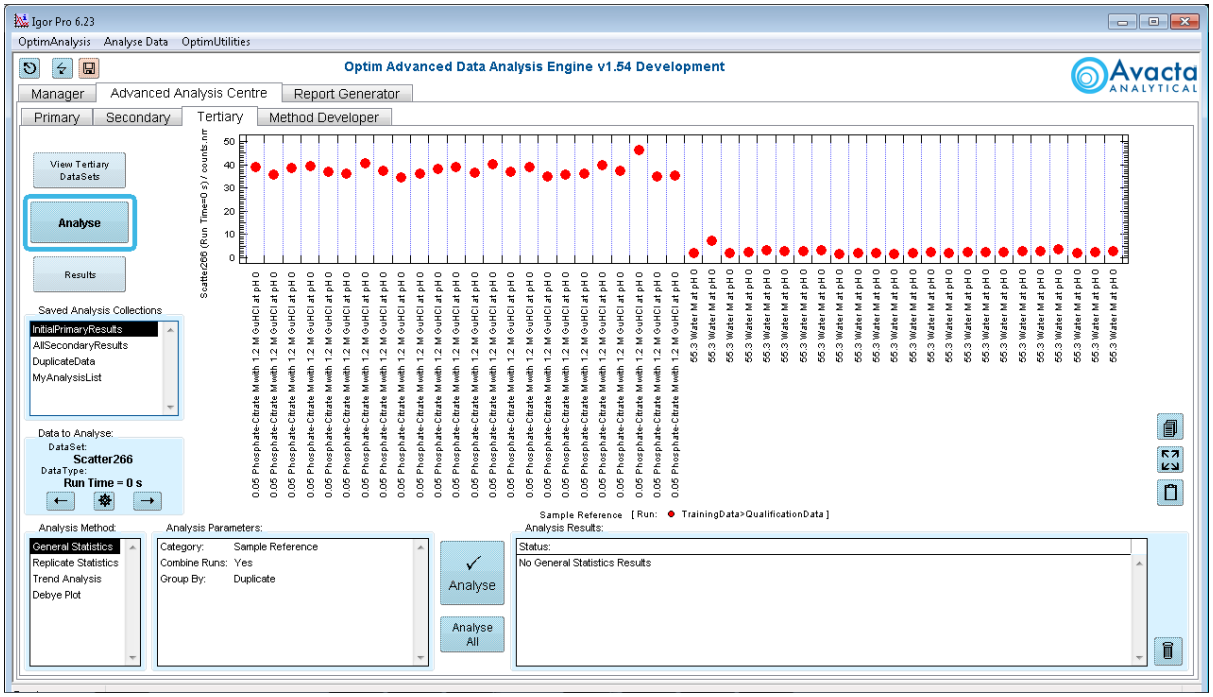
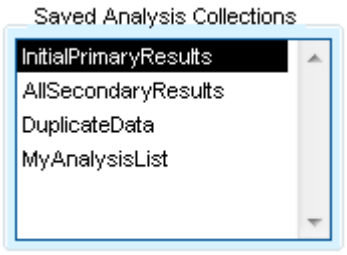


To analyse this data

- Select the Analysis Collection that you wish to analyse – in this case InitialPrimaryResults is selected.



- Select the method you wish to use – typically you would use
  - General Statistics with Duplicate Data
  - Replicate Statistics with Replicate Data
  - Trend Analysis with Trend Data
  - Debye Plot with Debye Data

- Set any analysis parameters you require (we will discuss these next)

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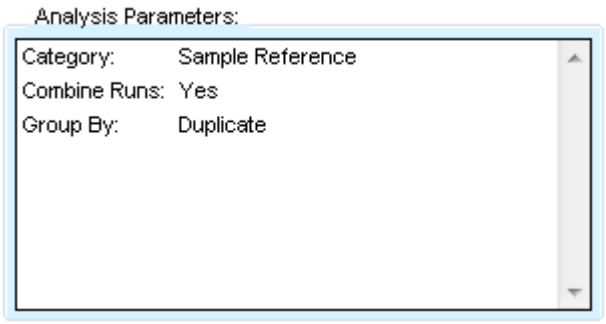
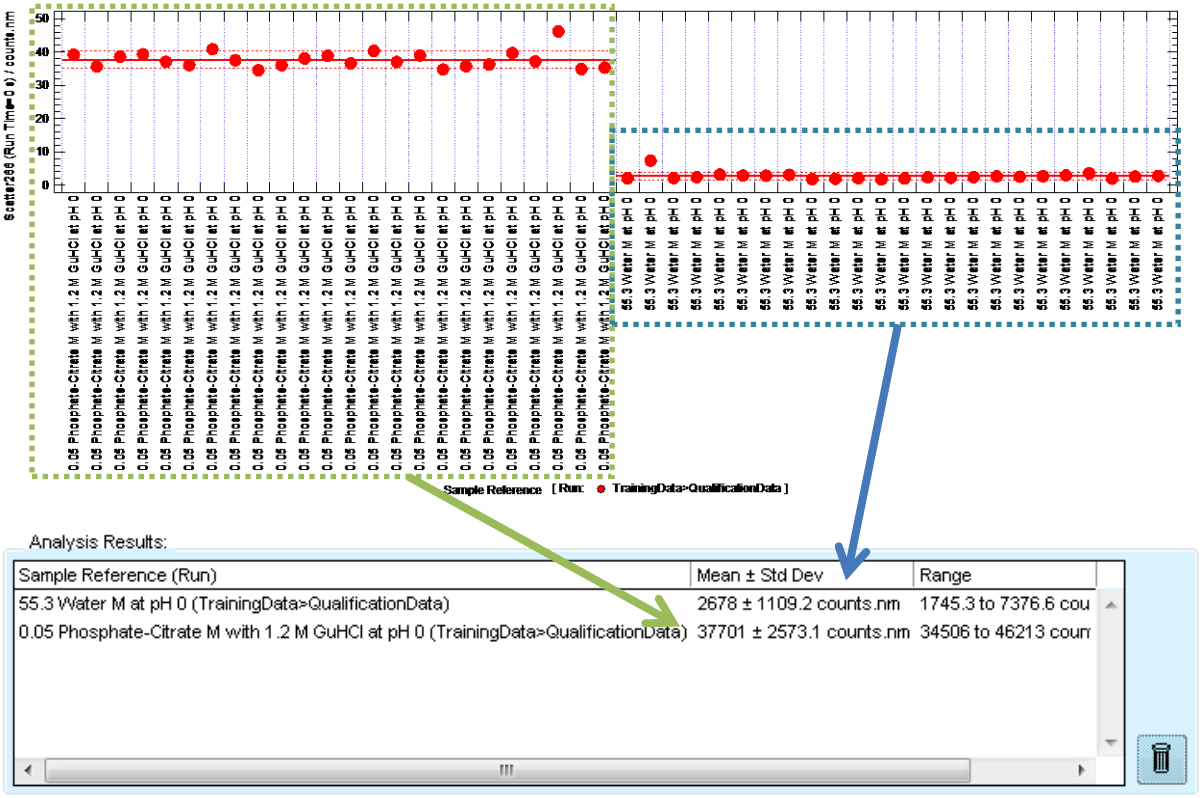


- Cycle through the datasets and data types until you have the one that interests you (as before, data in different runs will be in different colours).
- To analyse the trace on screen click ✓ANALYSE or ANALYSE ALL
- View the result on the graph and match the values shown in the Results box with the data displayed on screen


Analysis Results:
Sample Reference (Run)
Mean ± Std Dev
Range
55.3 Water M at pH 0 (TrainingData>QualificationData)
2678 ± 1109.2 counts.nm
1745.3 to 7376.6 cou
0.05 Phosphate-Citrate M with 1.2 M GuHCl at pH 0 (TrainingData>QualificationData)
37701 ± 2573.1 counts.nm
34506 to 46213 coun

Each of the tertiary analysis methods has parameters associated with it to allow tuning of the analyses. The available parameter depends on the particular method and these are detailed here.

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Method	Parameter	Options	Description
General Statistics	Category	Sample Reference Well	Changes the displayed category in the plot
	Group by	None	All data is analysed together
		Duplicate	Different duplicates within the collection are analysed separately
	Combine Runs	Yes	Grouping spans different runs
		No	Grouping in different runs are treated as different
Replicate Statistics	Category	Reference Number Sample Reference Well	Changes the displayed category in the plot
	Replicate Number	All	All replicates within the collection are analysed individually
		[x]	Only replicate number [x] is analysed
	Combine Runs	Yes	Grouping spans different runs
		No	Grouping in different runs are treated as different
Trend Analysis	Trend Parameter	Whatever available	A list of available numeric abscissas determined from the sample information provided
	Combine Runs	Yes	Grouping spans different runs
		No	Grouping in different runs are treated as different
	Match List	[ABC=XYZ,…]	List of differentiator sample parameters. For example if have trends in pH with two different concentrations of NaCl
	Data Number	1, 2, 3, …	When multiple results are available (for example two transition temperatures for a particular sample) this choice determines which to use
	Fit Type	Line	Fits a line to the data in the form y=mx+c
		Quadratic	Fits an offset quadratic of the form $(y-y_p)=c(x-x_p)^2$
		Denature	Fits a two state thermodynamic model to the data

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Debye Plot	Candidate	[ABC]	List of available protein candidates to plot
	Combine Runs	Yes	Grouping spans different runs
		No	Grouping in different runs are treated as different
	Match List	[ABC=XYZ,…]	List of differentiator sample parameters. For example if have trends in pH with two different concentrations of NaCl
	Wavelength	Auto	Determines wavelength of incident laser based on the available name of the primary analysis result
		[New]	Selecting this allows the user to enter any value
	Solvent R.I.	Auto	If possible will contain a list of known solvents with the refractive index automatically adjusted for wavelength
		Water	Uses the refractive index of water automatically adjusted for wavelength
		[New]	Selecting this allows the user to enter any value
	Solvent Well(s)	Mean	If multiple wells containing the solvent will calculate the mean value to use as the solvent in the calculations
		Median	If multiple wells containing the solvent will calculate the median value to use as the solvent in the calculations
		[Wells]	Allows user to choose an individual solvent well to use
	Reference	[WXY]	A list of available reference materials (for example water, toluene)
	Reference Wells(s)	Mean	If multiple wells containing the solvent will calculate the mean value to use as the solvent in the calculations
		Median	If multiple wells containing the solvent will calculate the median value to use as the solvent in the calculations
		[Wells]	Allows user to choose an individual solvent well to use
	Rayleigh ratio	Auto	If possible will contain a list of known solvents with the Rayleigh ratio automatically adjusted for wavelength
		Water	Uses the Rayleigh ratio of water automatically adjusted for wavelength
		[New]	Selecting this allows the user to enter any value
	dn/dc	0.185 ml/g	Value of this parameter
		[New]	Allows the user to enter any value
	Duplicate	None	How to group duplicate data

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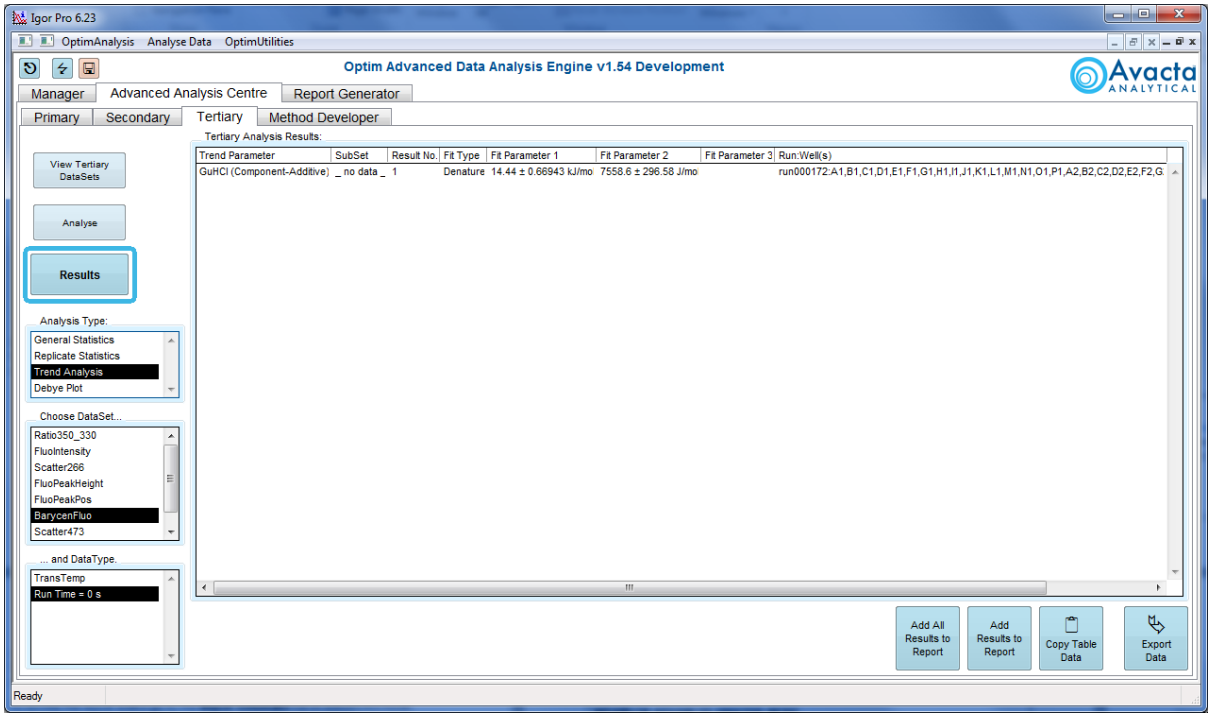
	average	Mean Median	

Viewing the results of the tertiary analysis

Ensure that the **Advanced Analysis** tab and the **Tertiary** sub tabs are selected and that RESULTS has been selected.



Choosing options from the Analysis Type, Dataset and Data Type will allow tables to be created containing the results of the tertiary analysis. These can be added to the report, copied and exported in the usual manner.

Generating a sample report

The Optim Analysis v1.5 software can be used to prepare a report that the user is able to edit, save, load and export.

To access the report editor go to the **Report Generator** tab in advanced mode.

A report will be viewable with the title "Optim Analysis Report" that has a header that shows the name of the current study.

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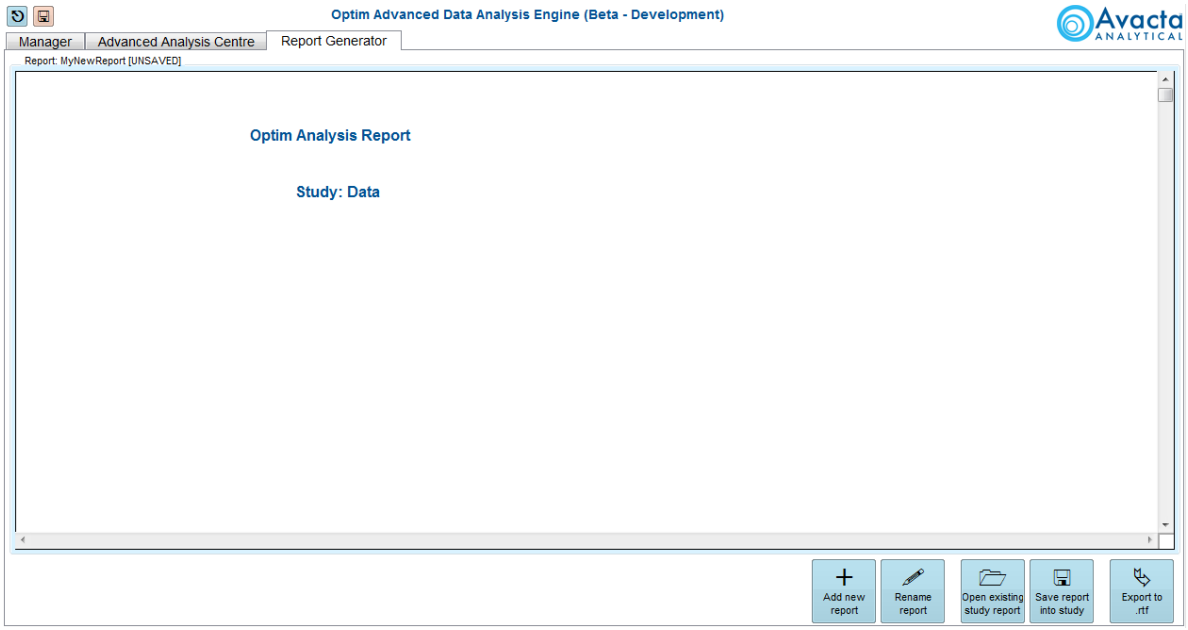

Within the report generator there are a number of buttons:

- +ADD NEW REPORT – will create a new report that is by default called "MyNewReport". There are no limits on the number of reports within the study.
- RENAME REPORT – will rename the current report.
- OPEN EXISTING STUDY REPORT – will load a report from the study folder that has previously been saved.
- SAVE REPORT INTO STUDY – will save a copy of the currently open report into the study folder.
- EXPORT TO .RTF – will export the currently open report into the study folder as a rich text format for editing in a word processing package of your choice.

Adding content to report

Within the analysis screens you will often come across a button that adds data to the report. By clicking on this button, the graph will be added to the report under a relevant section heading. The same can also be done with the results tables.

The report has a default style that constrains the graphs into categories of primary analysis, secondary analysis and tertiary analysis. As a result, graphs from the relevant sections go into each section of the report.

The report is editable however, and content can be moved around or deleted and text can be added.

IMPORTANT - YOU MUST SAVE THE REPORT AFTER YOU HAVE MANUALLY EDITED IT BY CLICKING SAVE REPORT INTO STUDY. OTHERWISE YOU WILL LOSE ANY TEXTUAL CHANGES YOU HAVE MADE IF YOU ADD FURTHER CONTENT THROUGH THE ANALYSIS WIZARD OR ADVANCED ANALYSIS MODE.

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Exporting report

By clicking on ↳EXPORT TO .RTF, a Rich Text Format variant of the report will be exported to the Studies folder. This can then be imported into Microsoft Word (or document processing package of preference) for further editing or importing into user standard templates.

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XVI. EDITING AND DEVELOPMENT OF ANALYSIS METHODS

One of the major new features of the analysis software v 1.5 is the ability to adapt and create new analytical methods to provide an optimal measure of the behaviour of measured samples.

The default methods

The default methods are typically geared towards the unfolding and aggregation of protein samples containing tryptophan.

Primary analysis methods

The default primary analysis methods consist of a method to analyse the static light scattering intensity called "ScatterMethod" and a method to analyse characteristics of the intrinsic fluorescence, "IntrinsicFluoMethod".

- Intrinsic Fluorescence
  - Calculates the **integrated intensity** of each spectrum in the wavelength range 280 – 460 nm. (The result of which is called FluoIntensity).
  - Determines an **intensity ratio** between the spectral intensity at 350 nm to that at 330 nm (called Ratio350_330).
  - Evaluates the expectation wavelength/**barycentric mean** of the spectra in the range of 280 – 460 nm. The barycentric mean is effectively the centre of mass (or a weighted average) of the fluorescence emission, and is given by $\lambda_m = \sum_\lambda f_\lambda \lambda / \sum_\lambda f_\lambda$ (called BarycenFluo).
  - Determines a **peak position** and a **peak height** by fitting a Lorentzian function to the fluorescence, initially centred at 340 nm with a width of 20 points and performing five iterations (called FluoPeakPos and FluoPeakHeight respectively).
- Scattering Intensity
  - Calculates the integrated intensity over the wavelength range 261 – 271 nm (called Scatter266).
  - Calculates the integrated intensity over the range 468 – 478 nm (called Scatter473).

There are also two further methods which are called "ScatterMethod_BufferSub" and "IntrinsicFluoMethod_BufferSub". These provide the same functionality as the other two methods but include an additional step where the software will match the provided characteristic information about individual sample wells to find an appropriate spectrum to subtract. For this to function correctly you need to:

- Have entered identical formulation information in the well in which the buffer will be loaded, but leave the Sample Details column empty.
- Have changed the reference column entry to TRUE.

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This will generate output results which have an appended "_B". For example, Ratio350_330 will be called Ratio350_330_B when a buffer spectrum has been subtracted.

The default methods in the analysis wizard are applied to the following default collections:

	Spectra Collections	
Analysis Methods	Buffer Subtraction: ON	Buffer Subtraction: OFF
ScatterMethod	SampleNoBufferSpectra	SampleSpectra
IntrinsicFluoMethod		
ScatterMethod_BufferSub	SampleBufferSpectra	_ None _
IntrinsicFluoMethod_BufferSub		

Secondary analysis methods

The default secondary analysis method includes a method to find the onset of the aggregation temperature and up to four melting points in the unfolding data. These can be edited as required or used as templates for the user's own methods.

The secondary analysis method that is used to determine transition temperatures (or melting temperatures if you are referring to thermal unfolding temperatures) is called Tm_method.

The method consists of two functions that occur in order:

1. Interpolate – to fill in any gaps in the primary analysis results data so that, even if a low temperature resolution scan is performed, it will find a result that is the most accurate value.
2. FindMultipleTm – this calculates the differential of the interpolated data and finds the position of peaks that exceed a specified signal-to-noise level of the smoothed differential to give the points of inflection in the curves. The sensitivity can be changed in the Method Development tab by changing either the smooth width or the signal-to-noise threshold.

An uncertainty is estimated by using the method described here.

The local noise of the unfolding curve (parameter versus temperature) is estimated by calculating the residual between a running a local smooth and the original data, calculating the local standard deviation of these residuals and correcting for the bias caused by only taking local samples. This has been verified to be correct with simulations.

The gradient of the unfolding curve at this point is determined by the height of the first differential.

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The 'uncertainty' in the transition temperature is then estimated as: noise / gradient.

The data is not fitted to any particular function, so determining the uncertainty in a fit parameter from a chi-squared or similar statistic is not possible If an attempt were made to fit the data to a particular functional form, then theoretically the error in certain fit parameters could be very well determined, but this is only true if the functional form were a true representation of the shape of the data, which, in practice, is rarely the case. The secondary analysis method that determines the onset temperatures, whether the onset of unfolding, or T_{agg}, the onset of aggregation is more complicated.

It consists of five functions:

1. Interpolate – to fill in any gaps in the primary analysis results data so that, even if a low temperature resolution scan is performed, it will find a result that is the most accurate value.
2. Smooth – performs a five point median smooth of the interpolated data to reduce the effect of spikes in the data, due to dust etc, causing an artifactual anomalous result.
3. Differentiate – differentiates the smoothed data to reveal the points where the signal starts to increase most rapidly.
4. Heaviside – applies a Heaviside function to the differentiated data so that the cumulative signal level is analysed. This removes problems that can be caused if a sudden onset is followed by a sudden decrease in signal.
5. FindEdgeLoc – finds the location of a positive going edge in the output of the Heaviside function. The default is to find an edge that is 10% of the maximum intensity. The sensitivity can be edited by changing the function's fraction parameter in the method developer.

For isothermal data, where kinetic rate constants are required the isothermal_method will fit a mono-exponential function to the data. This will return the rate constant calculated from the data.

Creating and editing methods

New methods can be created in a very simple manner using the Optim Analysis software. The application of methods is grouped by analysis level in the software so methods are developed specifically for each stage of the analysis process. To create a new or edit an existing analysis method you should navigate to the **Advanced Analysis Centre** tab and the **Method Developer** sub-tab and ensure that EDIT is selected.

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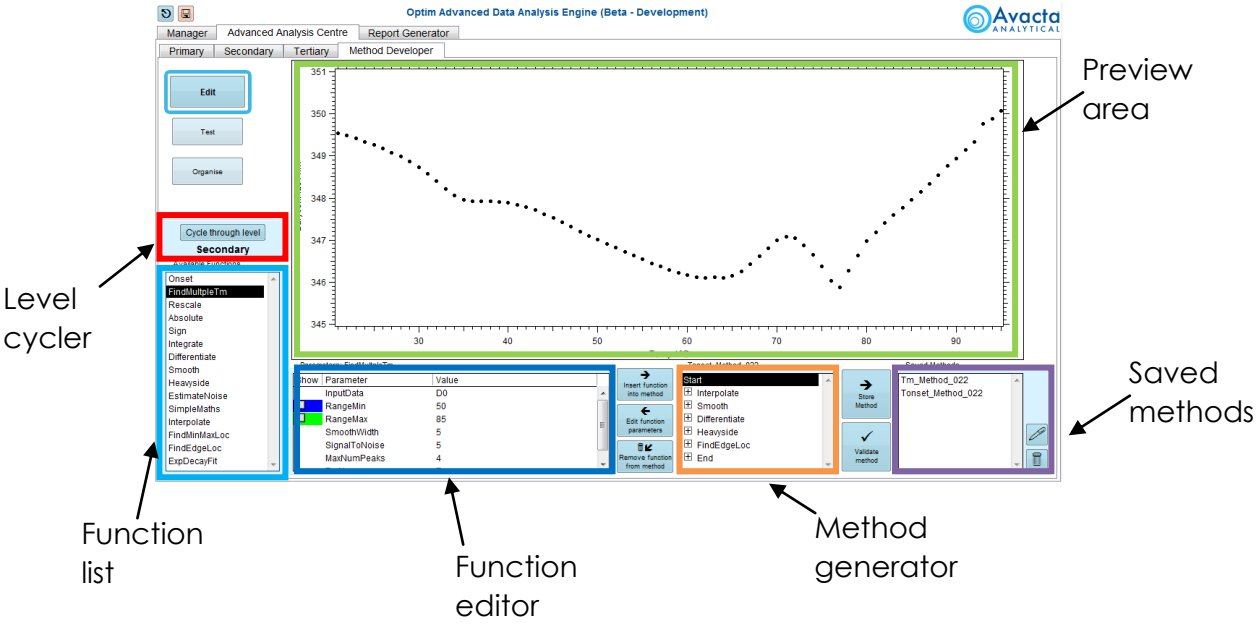

The screen is divided up into six different regions. When developing new methods this is the direction of work flow through the system:

- **Preview area** – the data for which a method is being developed is shown here.
- **Level cycler** – this shows the analysis level that you are editing functions for. Clicking CYCLE THROUGH LEVEL allows you to change from primary analysis functions to secondary and tertiary analysis functions.
- **Function list** – this shows a list of the available functions for the analysis level that you are looking at. Details about these functions is given later.
- **Function editor** – here the parameters relating to a particular function can be edited.
- **Method builder** – here the functions are combined to create an analysis method.
- **Saved methods** – here the methods are saved for use in the software. Methods can be saved by clicking ➔STORE METHOD, deleted by clicking 🗑, and renamed with ✎.

Saved methods can be loaded into the method developer by double clicking on them in the Saved Methods box.

Once the method is loaded into the method developer, you can insert new functions or edit existing functions.

How to select data for Method Development:

- Primary
    - To display a particular *spectrum* in the method developer then you should select it from the Select Spectra list in the **Primary** sub-tab of the **Advanced Analysis Centre** (highlight the desired spectrum so that it

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appears in the graph) *and* ensure that no spectra collections are highlighted).
    - Alternatively, you can select multiple spectra or a spectra collection, and the *first* spectrum of this group will be displayed in the preview area of the Edit tab. The rest of the selected spectra can be scrolled through using the same method in the Test tab.
- Secondary
    - The *first* dataset of the selected Analysis Collection will be displayed in the Edit tab. The remaining datasets can be scrolled through in the Test tab.

Editing the default primary analysis fluorescence method to include a background subtraction

Follow this example. Load the *IntrinsicFluoMethod* primary analysis method into the method developer. You will see that there are four functions as part of this method as discussed in the previous section.

NB When spectra are acquired in the client software, the instrument automatically subtracts a 'dark count'. For the purposes of this tutorial, the example dataset has spectra which have not had the dark count subtracted.

To add a background subtraction select *Start* in the **method generator** and *SubBackgroundRange* in the list of functions. Click ➔INSERT FUNCTION INTO METHOD to add this function to the top of the list in the method.

This function will take the input spectra, subtract an average of the signal in the range specified by the *RangeMin* and R*angeMax* parameters and create an output wave in a different dataset. RangeMin and RangeMax can be changed by typing different values in the parameter editor or selecting the line in the preview area, dragging it to a new position, right clicking and selecting *Set*.

When a parameter has a checkbox next to it, ticking the box will display the parameter in the preview area in the colour specified. So *RangeMin* will be displayed as a blue line and *RangeMax* as a green line.

Parameters: SubBackgroundRange

Show	Parameter	Value
	InputData	D0
	OutputData	D1
☐	RangeMin	280
☐	RangeMax	290

Datasets in the Optim Analysis software are called Dx where x is a number starting at 0 for the highest level dataset. All the functions that are currently in the method act on data that is in the dataset D0, the results of the function become dataset D1.In

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the method generator, select *IntensityRatio* and click ←EDIT FUNCTION PARAMETERS. In the function editor you will see that the input data for this function is data in dataset D0. Click on the value in the editor and change it to D1. To insert this function back into the method click →INSERT FUNCTION INTO METHOD.

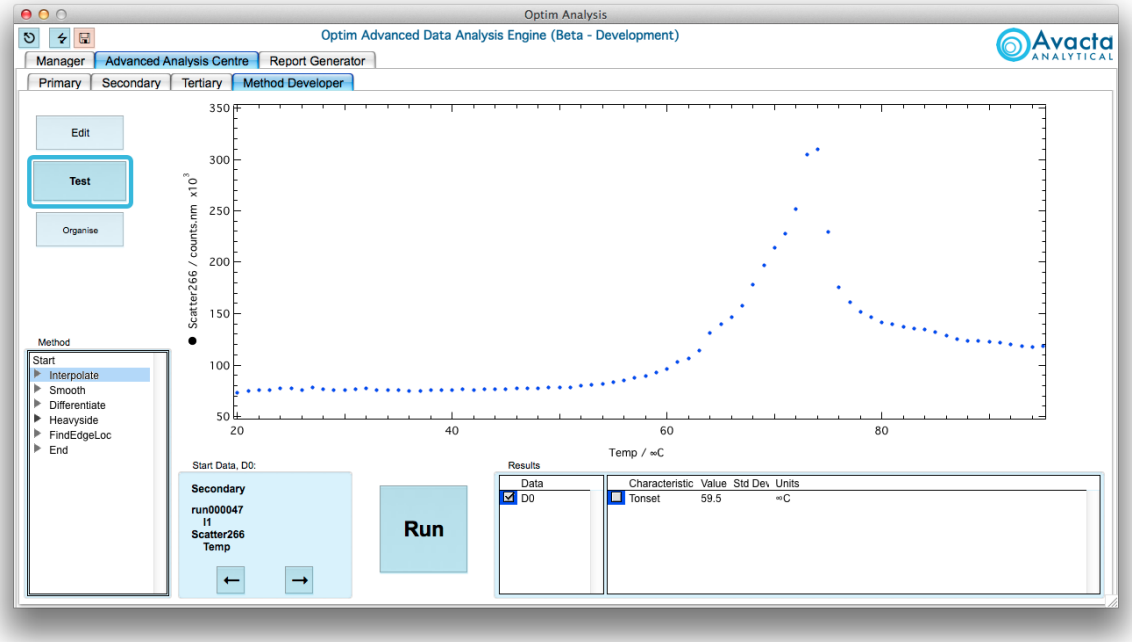
⚠IMPORTANT – ORDER IS IMPORTANT IN THE METHOD. THE FUNCTION WILL BE ADDED TO THE METHOD AFTER THE FUNCTION THAT IS CURRENTLY SELECTED IN THE METHOD GENERATOR. ENSURE THAT YOU DO NOT INSERT THIS FUNCTION INTO THE METHOD BEFORE DATASET D1 IS CREATED BY THE BACKGROUND SUBTRACTION FUNCTION.

Repeat this then for each function in the method so that they all act on the background subtracted spectra. Save the method by clicking →STORE METHOD and calling the method *IntrinsicFluBackground*.

To change the results that are presented to the user from the analysis method select *End* in the method generator and click ←EDIT FUNCTION PARAMETERS. You will see the Function editor changes to list all of the available outputs with a checkbox. Those method outputs that are not checked are hidden from the user. To enable testing of the method and the display of the background subtracted spectrum check the box next to D1 and click ✓→UPDATE OUTPUT.

Testing a method

The method that is being edited can be tested against the data that is displayed in the Preview area. To test the currently loaded analysis method you should navigate to the **Advanced Analysis Centre** tab and the **Method Developer** sub-tab and ensure that TEST is selected.



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The method that you are testing is displayed in this area for reference purposes only and cannot be edited from this tab.

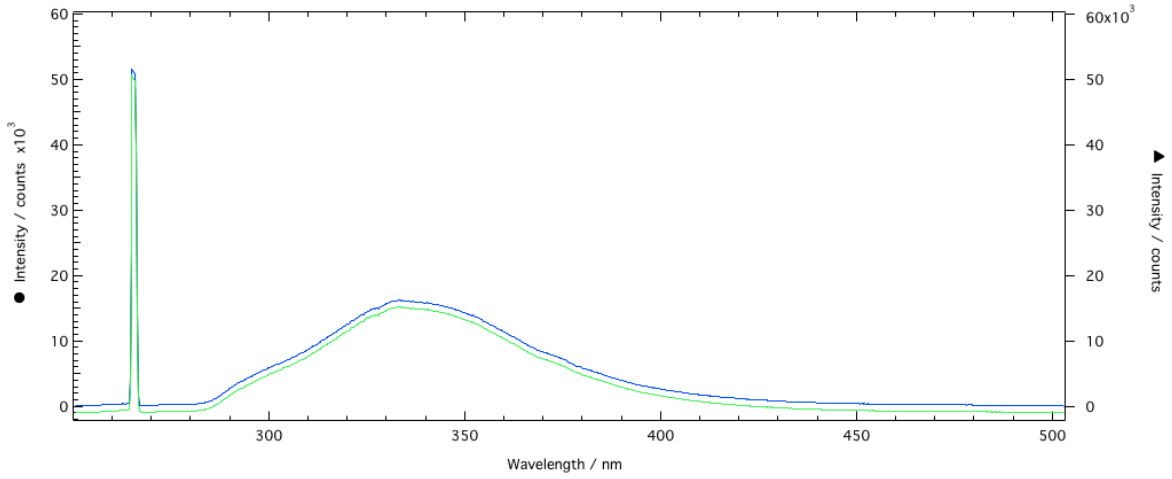
The data displayed in the preview area will be the selected data in the relevant analysis sub-tab. See above for instructions on how to select the test data. To test the method against a different spectrum or curve in the collection click either ← or →. Click the RUN button to test the method and display the resulting datasets chosen by the checkbox in the Results dialog. Calculated values that are output from the analysis method are displayed in the results box.

Results

Data	Characteristic	Value	Std Dev	Units
☑ D0	FluoIntensity	1.1262e+06		counts.nm
☑ D1	Ratio350_330	0.91193		
	BarycenFluo	345.14		nm
	FluoPeakPos	337.03	0.013125	nm
	FluoPeakHeight	16074	285.47	counts

In this case datasets D0 – the original data, and D1 – the background subtracted data are available and displayed. The calculated results are shown with units and an uncertainty if available.

With both D0 and D1 checkboxes ticked, the Preview area displays both traces.



TIP – If you expected to see more output datasets, ensure that you have enabled the output for the dataset you want in the *End* function of the method.

Method management

Methods that have been created in your study can be added to the default methods, and *vice versa*, so that they can be used in the wizard mode and in other studies.

Navigate to the **Advanced Analysis Centre** tab and the **Method developer** sub-tab and ensure that ORGANISE is selected.

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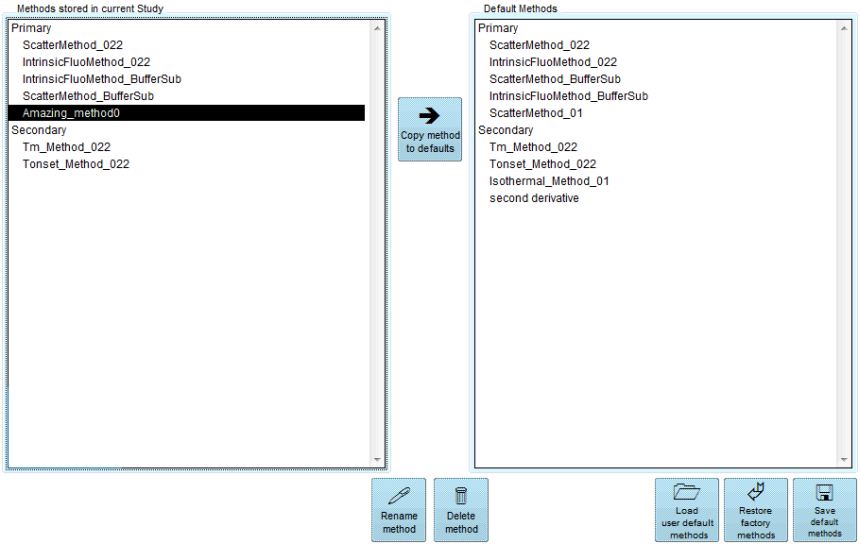

By selecting different methods that are in the study they can be added to the default methods by clicking ➔COPY METHOD TO DEFAULTS. By selecting a default method clicking ➔COPY METHOD TO STUDY a default method can be added to the current study.

The default methods are present in all newly created analysis studies. If you have added a new method to the default methods you can click 🖫SAVE DEFAULT METHODS and it will appear in all new studies.

If at any point you wish to restore the user default methods you can click 🗁LOAD USER DEFAULT METHODS. To restore the default methods to the factory settings click ⮌RESTORE FACTORY METHODS.

The factory default methods are hard coded into the analysis software, so as new versions are released and new methods added they can be added to your studies by restoring factory methods and manually copying them across to your studies.

Definition of standard functions included in the Optim Analysis software

In this part of the handbook you will find a definition of all functions and their parameters that are included as standard with the Optim Analysis software.

For any given Method, the input dataset is labelled 'D0'. This can be a spectrum, temperature unfolding curve, or anything else. A Function can take this 'D0' as input, and other parameters, and output either a new dataset (say 'D1') and/or some characteristic values or Results. Subsequent Functions can take any of the datasets as input (i.e. 'D0' or 'D1', etc) and other parameters, some of which may be previously determined characteristic values from within the same Method. Finally, a list of the characteristic values or results, determines results to be stored as Output from this Method.

The following is a list of available Functions:

SubBackgroundRange

InputData Spectrum to be acted upon.
OutputData Resultant Spectrum.

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RangeMin Minimum value of range.
RangeMax Maximum value of range.

This calculates the mean (y) value of the data in the specified range, and subtracts this from all data points in the spectrum.

SubBackgroundRange2

InputData Spectrum to be acted upon.
OutputData Resultant Spectrum.
RangeMin1 Minimum value of range 1.
RangeMax1 Maximum value of range 1.
RangeMin2 Minimum value of range 2.
RangeMax2 Maximum value of range 2.

This fits a straight line to the data within the two specified ranges, simultaneously, and subtracts this line from the whole spectrum.

This is useful, for example, in calculating the integrated intensity of a peak above a sloping baseline, where one uses this function with ranges either side of the desired peak, then performs an integrate area function on the resultant.

Rescale

InputData	Spectrum to be acted upon.
OutputData	Resultant Spectrum.
OffsetValue	Value to Add to all points (this is performed before the multiplication).
ScalingFactor	Value to Multiply to all points (this is performed after the addition).

The following calculation is performed on each point in the input dataset:

Output = (Input + Offset) * ScalingFactor

Absolute

InputData Spectrum to be acted upon.
OutputData Resultant Spectrum.
Absolute +1 to force values to be positive, -1 for negative.

This function takes each y-value of the input data, and converts it to the Absolute (i.e. the sign of the value is forced to be either positive or negative, depending upon the value of Absolute) of this value in the output dataset.

Sign

InputData Spectrum to be acted upon.
OutputData Resultant Spectrum.

This function takes each y-value of the input data, and converts it to the Sign (i.e. -1 if negative, +1 if not negative) of this value in the output dataset.

Integrate

InputData Spectrum to be acted upon.
OutputData Resultant Spectrum.

This function performs a numeric integration of input data (spectrum).The first data point of the OutputData has value zero, and there is one more data point in the OutputData than in the InputData.

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Differentiate

InputData	Spectrum to be acted upon.
OutputData	Resultant Spectrum.

This function performs a numeric differentiation of input data (spectrum).There is one less data point in the OutputData than in the InputData.

Smooth

InputData	Spectrum to be acted upon.
SmthPnt	Number of data points to use for the smooth. Must be Odd (will round down to odd if not) and minimum value is 3.
Method	Smooth Method options: Box : simple mean. Median : middle. Binomial Savitzky-Golay : weighted means.
End effect	Bounce.
OutputData	Resultant.

This function performs a running Smooth of the input data (spectrum).

Heavyside

InputData	Spectrum to be acted upon.
Polarity	Positive : running maximum. Negative : running minimum. Auto : function tries to work out which way the trend is going.
OutputData	Resultant.
AutoPolName	Name of the output - value will 1 for positive, or 0 for negative.

This function performs a Heaviside operation on input data (spectrum). Each data point in the output determined from all points in the input up to the point under consideration. For a positive polarity, each value of the output is the maximum of all previous values.

EstimateNoise

InputData	Spectrum to be acted upon.
SmthPnt	Number of data points to use for initial smooth.
StdDevPnts	Number of data points to use for standard deviation.
OutputData	Resultant.

This function estimates the noise at each point in the InputData. It performs a running smooth, then calculates the residual between this smoothed dataset and the original dataset. It then calculates the local standard deviation of these residuals, and corrects this for the size of the sample to give an estimate of the local standard deviation at each point.

SimpleMaths

InputData	Spectrum to be acted upon.
AuxInputData	Spectrum to be added, subtracted (eg a buffer spectrum), multiplied, or divided (denominator).
OutputData	Resultant.
Function	[InputData] +/-/÷/x [AuxInputData].
MatchMethod	Method used to match x-axis.

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This function performs the given mathematical function on the two input datasets. The x-values of the OutputData are the same as InputData. The AuxInputData's x-scale is adjusted to match those of the InputData so that the point-by-point maths can be performed. The Match Method options are "Interpolate" where the shape of the data is preserved, and "Preserve Area" where the integral of the data is preserved (an example of this is to preserve photon counts in a spectrum when changing the x-scale).

Interpolate

InputData	Spectrum to be acted upon.
OutputData	Resultant.
Start	Value of first point, or 'Auto'.
End	Value of last point, or 'Auto'.
NumPoints	Number of data points, or 'Auto'. This overrides Spacing
Spacing	Spacing between data points, or 'Auto'.
Rounding	Round Auto parameters to 'nice' values.

This function produces an interpolated version of the InputData where the x-scale is adjusted to be a uniform spacing. The various options allow the user to control the details of the x-scale limits and spacing.

FindMinMaxLoc

InputData	Spectrum to be acted upon.
MinOrMax	Whether looking for minimum or maximum.
MinMaxName	Name of Results File.

This function gives as the output the x-value corresponding to the maximum or minimum y-value.

FindEdgeLoc

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
Fraction	Fraction up the edge for edge location (usually 0 < frac < 0.5)
EdgeName	Name of Results File.

This function gives as the output the estimate of the start of an edge in the InputData. This is roughly the Fraction of the edge height. The data are interrogated within the given range only.

IntegrateArea

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
IntegrationName	Name of Results File.

This function determines the area under the InputData between the given limits.

IntensityRatio

InputData	Spectrum to be acted upon.
NumeratorValue	Centre value for integration, numerator of ratio.
DenominatorValue	Centre value for integration, denominator of ratio.
FullWidth	Width for each integration.
RatioName	Name of Results File.

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This function calculates the integrated area under the DataSet for the two regions whose centres are given and with the given Width. A ratio of the two areas (Numerator / Denominator) gives the output value.

BarycentricMean

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
BarycentricName	Name of Results File.

This function determines the mean x-value within the given range. This means each x-value is weighted by the corresponding y-values, and the mean of these determined.

PeakFit

InputData	Spectrum to be acted upon.
RangeCentre	Centre of Fit range.
RangeWidth	Width of Fit range.
FitMethod	Fit Method (Gaussian/Lorentzian/Quadratic/LogNormal).
Iterations	Number of Iterations (move centre).
PeakPositionName	Name of Results File.
PeakHeightName	Name of Results File.

This function fits the data within the given range to the given curve (peak) shape. The parameters corresponding to peak position and peak height are output.

ExpDecayFit

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of Fit range.
RangeMax	Maximum of Fit range.
DecayConstantName	Name of Results File.

This function fits the data within the given range to an offset exponential decay. The decay constant (1/e point) is output.

SimpleTm

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
SmoothWidth	DataPoints for Median Smooth.
TmName	Name of Results File.

This function calculates the differential of the InputData, after having performed a simple box smooth, and then determines the maximum of this to estimate the Tm.

FindPeakTm

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
SmoothWidth	DataPoints for Median Smooth.
MinLevel	Minimium level for peak.
TmName	Name of Results File.

This function calculates the differential of the InputData, and looks for a peak in this data between the desired ranges.

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FindMultpleTm

InputData	Spectrum to be acted upon.
RangeMin	Minimum value of range.
RangeMax	Maximum value of range.
SmoothWidth	DataPoints for Median Smooth and Averaging.
SignalToNoise	Minimium level for peak.
MaxNumPeaks	Maximium number of peaks.
TmName	Name of Results File.

This function calculates the differential of the InputData and searches for peaks within the given range. It estimates the uncertainty in these Tm by estimating the local noise at these points.

Onset

InputData	Spectrum to be acted upon.
StartPoints	Number of points to start search.
Threshold	S/N threshold.
Sensitivity	Chi-squared multiplier before onset deemed valid.
TonsetName	Name of Results File.

This function tries to determine an Onset point by determining the point beyond which the data no longer fit to a straight line.

MatchCharacteristic

InputData	Spectrum to be acted upon.
AnalysisType	Class of Analysis.
ListName	Name of List of Data to search for match.
OutputData	Resultant.
CharAbsName	Name of Abscissa Characteristic (for closest match). If empty, then this parameter is ignored.
CharAbsValue	Value Characteristic. If empty, then the value is taken from InputData.
CharName#	Name of Characteristic. If empty, then this parameter is ignored.
CharValue#	Value Characteristic. If empty, then the value is taken from InputData.

This function returns OutputData whereby this data is one of the datasets in the given lists (collections) of datasets, and whose characteristics match those supplied. The Abscissa Characteristic, if supplied, is not required to be an exact match, but instead the closest match.

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APPENDICES

XVII. RISK ASSESSMENTS

General usage

1) Sample Loading Door

CAUTION - The user may trap their fingers/hand in the door opening.

Who might be harmed, and how?	User – Sliding door may trap or crush hand or fingers.	
What is being done already?	The door is opened/closed automatically. The risk of this happening with sufficient force to cause harm is very low. Correct operation of the door is to not leave hands/fingers inside the opening when the door is closing. With correct operation, the risk is very low.	
What further action is necessary?	The user needs to be trained in the appropriate operating procedures.	
By whom? When?		Done?

2) Sample stage motion

CAUTION – The user may trap/crush their fingers/hand with the sample X-Y stage.

Who might be harmed, and how?	User – If the X-Y stage moves when the user has their hand in the way.	
What is being done already?	The X-Y stage should not move when the door is open. This will prevent this from happening. The stage moves slowly (maximum rate ~1cm/s) that the user would have sufficient time to remove their hand/fingers before significant harm could be done, if the stage were to move with the door open, and the user has their hand inside. The risk of this happening is therefore very low.	
What further action is necessary?	No further action is necessary as the risk is very low.	
By whom? When?		Done?

3) Operation without side panels

DANGER – Electrical Shock Hazard. High voltage contacts may be exposed when the instrument panels are not in place.

Who might be harmed, and how?	User – Severe electric shock can result from operating the instrument without its panels in place by touching electrical terminals inside the instrument.	
What is being done already?	All electrical terminals can only be accessed by removing the instrument panels. The user is advised to not remove the panels. Severe electric shock can result from operating the instrument without its panels in place. Do not remove the instrument panels. Correct operation of the instrument is to only operate it when the panels are in place. With correct operation, the risk is very low.	
What further action is necessary?	The user needs to be trained to not remove the panels.	
By whom? When?		Done?

4) Mains Electricity

DANGER – Electrical Shock Hazard. The metal outside of the instrument may become live.

Who might be harmed, and how?	User – Severe electric shock can result from touching live electrical conductors.	
What is being done already?	All live electrical wires inside the instrument are firmly attached to terminals. The risk of these becoming detached is low. If any were to become detached, this would be detected by the power management system inside the instrument or by the incorrect operation of the instrument. All panels and framework that are at risk of becoming in contact with a detached live wire are earth bonded. The risk to the user is therefore very low.	
What further action is necessary?	The user needs to be trained to not remove the panels.	
By whom? When?		Done?

5) Hot Plate

WARNING – Physical Injury Hazard. The plate where the samples are located may become hot during operation.

Who might be harmed, and how?	User – The temperature of the heating stage may routinely be heated to ~100°C. The user touching this hot plate may receive burns to their hands/fingers.	
What is being done already?	The user should allow sufficient time for the stage to cool before moving or changing the samples. The instrument should prevent the door from being opened whilst the stage is hot. With correct operation of the instrument, the risk to the user is therefore very low.	
What further action is necessary?	No further action is necessary as the risk is very low.	
By whom? When?		Done?

6) Lasers

DANGER – Laser Hazard. Direct or indirect exposure of the laser beam to the eyes or skin may cause harm.

Who might be harmed, and how?	User – during sample analysis the samples are subject to exposure to one or more laser beams. The user may be harmed if their hands/fingers intercept this beam.	
What is being done already?	In normal operation, the shutters in the laser beam will only operate when the sample loading door is closed. With the panels in place and the door closed, it is highly unlikely for any part of the user to be able to intercept the laser beams. With correct operation of the instrument, the risk to the user is therefore very low.	
What further action is necessary?	No further action is necessary as the risk is very low.	
By whom? When?		Done?

7) Relocation

CAUTION Physical Injury Hazard. Moving or lifting the instrument and/or associated computers may result in injury to the musculoskeletal system.

Do not attempt to move or lift the instrument without the assistance of others, the use of appropriate moving equipment and proper lifting techniques.

Who might be harmed, and how?	Service engineer/other helpers - inappropriate lifting techniques may result in a painful back or other musculoskeletal disorder.	
What is being done already?	The service engineer is trained in proper lifting techniques. Where assistance is required from others, they should also have received appropriate training. With the use of proper techniques the risk is very low.	
What further action is necessary?	If assistance is required from others, the service engineer should ensure they are properly trained. With the use of proper techniques the risk is very low.	
By whom? When?		Done?

XVIII. GLOSSARY OF TERMS

Physical things:

Micro-Cuvette Array (MCA) – The black/blue anodised sample-holder that contains an array of 16 square or round quartz micro-cuvettes.

> **Cuvette Holder -** the central black unit into which the micro-cuvettes (round or square) are inserted. (The cuvette holder has one or two parts depending on the cuvette type).
>
> **Frame (blue)** – the frame is made of two hinged caps, each of which will hold a rubber **seal**. The cuvette holder slides into the **holding cap** of the blue anodised frame and is held in place by the **closing cap** and **clip**.

Cuvette – A round or square micro-cuvette.

Well – An alternative name for "cuvette", used for descriptive purposes to represent the chamber that contains the formulation.

Sample - The protein or primary molecule of interest.

Formulation – The mixture of sample with buffer and optional additives which is inside a cuvette (or well) of the MCA.

Hot Plate – The copper plate with brass spigots to mount MCAs. The plate heats the samples.

Stage – The hot plate is mounted upon a stage. The stage moves by motor control or as a manual drawer.

Software things:

Capture – A single shot of the CCD camera, a raw image that can be processed to produce a spectrum.

Single Shot – This command tells the camera to take a capture (using the current settings). This capture will not be saved to the database.

Run - A succession of captures with user-defined settings (automatically saved to the database).

Measurement – A measurement that can be performed on the sample (e.g. UV fluorescence or blue light scattering). Measurements are defined by the choice of experiment.

Experiment – A template for a standard operating procedure (SOP) that defines the combination of measurements that will be performed on the sample.

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Project – The user selects a project at the Login stage of the Client Software. This gives them access to all raw spectra, analysis results and reports for a given study. Generally a new Project is created for each molecule of interest.

Study – A folder of work inside the Analysis Software, where spectra can be viewed, grouped, analysed and reports can be written and saved.

Calibrations – A set of mathematical functions that are used to process the raw capture image into a spectrum (e.g. Background subtraction, wavelength calibration, device response calibration).

Spectrum – A calibrated image from the CCD camera that gives information about the intensity of light at different wavelengths.

Collection – A user-defined or default selected group of either spectra or datasets in the Analysis Software that will be carried forward to the next step in the analysis process.

Ordinate – The property of the data that will be plotted on the y-axis of the graph.

Abscissa – The property of the data that will be plotted on the x-axis of the graph.

Primary Analysis – Analysis of each corrected spectrum as defined by the selected macro. This level of analysis yields a single plot for each cuvette/well and for each analysis technique (e.g. An unfolding curve such as fluorescence peak position versus temperature).

Secondary Analysis – Analysis of the unfolding curve (primary results) for each of the cuvettes/wells and analysis techniques to identify transition points (e.g. melting point, T_m).

Tertiary Analysis – Multi-cuvette analysis. Comparing the transition points (secondary results) in different cuvettes (e.g. Effect of different formulations or consistency between replicates).

About Avacta Analytical

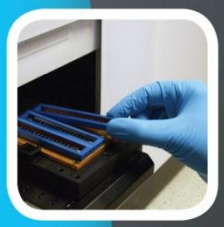
Avacta Analytical provides innovative analysis and detection solutions to the biopharmaceutical, pharmaceutical and healthcare industries through leveraging its broad ranging expertise in analytical techniques and protein science.

Avacta Analytical's unique skill lies in making advanced analytical technology more broadly accessible through a range of market-centric products and services. Our primary focus is to equip pharmaceutical developers and manufacturers with tools to get their products to market more quickly, at reduced cost, and to optimise the critical-to-performance properties of these new drugs.

Avacta Analytical's products and services make it possible for drug developers to analyse their compounds in much greater detail at an earlier stage in the drug development pipeline than is now possible, thereby providing timely identification of problems that can cause costly late stage failures or poor product performance.

Avacta Analytical is a wholly-owned subsidiary of Avacta Group plc, and occupies its own purpose built laboratories and manufacturing facility.

Avacta Analytical is ISO9001 registered.



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